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ASSESSMENT REPORT
ON NITROSAMINES



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ON
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PREFACE

This document was prepared by a task force directed by Dr. F. G. Hueter, Director, Criteria and Special Studies Office, Health Effects Research Laboratory, U.S. Environmental Protection Agency (EPA), Research Triangle Park (RTP), N.C. The objective of the task force was to review and evaluate current knowledge on nitrosamines in the environment as related to possible deleterious effects on human health and welfare. The document is not an exhaustive scientific review of the subject but is an attempt to place the subject in perspective relative to the need for control of nitrosamines.

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All comments and criticisms have been reviewed and incorporated in the document where deemed appropriate.

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LIST OF ABBREVIATIONS AND SYMBOLS

⊙	Benzene, or phenyl group	hν	Used to denote photon or photolytic energy
B.W.	Body weight	in.	Inch
°C	Degrees Celsius	K	Rate constant for a reaction (also K ₁ , K ₂ , etc.)
¹⁴ C	Radioisotope of carbon	kg	Kilogram
cm ³	Cubic centimeter	km	Kilometer
CO ₂	Carbon dioxide	KOH	Potassium hydroxide
CoA	Coenzyme A	L.D ₅₀	Dose lethal to 50 percent of recipients
(CH ₃) ₂ NH	Dimethylamine (DMA)	m	Meter
(CH ₃) ₂ N-NO	Dimethylnitrosamine (DMN)	M	Molar
C-N=O	C-nitroso; a nitroso group attached to a carbon atom	m ³	Cubic meter
CaCO ₃	Calcium carbonate	mg	Milligram
CaSO ₄	Calcium sulfate	Mg	Megagram
[t(CH ₃) ₂ N-CS-S] ₂ Zn	Ziram (a fungicide)	min.	Minute
[t(CH ₃) ₂ N-CS-S] ₂ Fe	Ferbam (a fungicide)	ml	Milliliter
DEN	Diethylnitrosamine	MS	Mass spectrometry
DMA	Dimethylamine	MT	Metric ton
DMN	Dimethylnitrosamine	N or N ₂	Nitrogen
DNA	Deoxyribonucleic acid	¹⁵ N	Isotope of nitrogen
EPA	U.S. Environmental Protection Agency	NADPH	Reduced (hydrogenated) nicotinamide adenine dinucleotide phosphate
°F	Degrees Fahrenheit	NaNO ₂	Sodium nitrite
Fe	Iron	NCS ⁻	Thiocyanate ion
ft	Foot	ng	Nanogram
g	Gram	NH ₂	Used to denote amines or amino group
GC	Gas chromatography	NH ₃	Ammonia
G.I.	Gastrointestinal	NH ₄ ⁺	Ammonium ion
GLC	Gas-liquid chromatography	(NH ₄) ₂ SO ₄	Ammonium sulfate
ha	Hectare	nm	Nanometer
HCl	Hydrochloric acid	N-6-MI	N-nitrosohepta-methyleneimine
HCN	Hydrogen cyanide	N-N=P	N-nitroso; nitroso group attached to a nitrogen atom
HNO ₂	Nitrous acid		
H ₂ NO ₂ ⁺	Nitrous acidium ion		
HONO	Nitrous acid		
H ₂ O	Water		
HPLC	High-pressure (or, high-performance) liquid chromatography		

NO	Nitric oxide	pK _a	Log of the reciprocal of the dissociation constant for an acid
N=O	Nitroso group	pph	Part per billion
NO ₂	Nitrogen dioxide	ppm	Part per million
NO ₂ ⁻	Nitrite ion	ppt	Part per trillion
NO ₃ ⁻	Nitrate ion	psi	Pound per square inch
N ₂ O	Nitrous oxide	R	Functional group (also R', R ₁ , R ₂ , etc.)
N ₂ O ₃	Dinitrogen trioxide (nitrous anhydride)	RNA	Ribonucleic acid
NOBr	Nitrosyl bromide	RTI	Research Triangle Institute; RTP, N.C.
NOCl	Nitrosyl chloride	RTP	Research Triangle Park, N.C.
NO ₃ -N	Nitrate nitrogen	S	Sulfur
NO _x	Nitrogen oxides	scfm	Standard cubic foot per minute
NOX	Nitrosyl halide	sec	Second
NPip	N-nitrosopiperidine	TEA	"Thermal energy analysis"
NPro	N-nitrosoproline	TERC	Thermo Electron Research Center; Waltham, Mass.
NPyr	N-nitrosopyrrolidine	μg	Microgram
O ₃	Ozone	UMDH	Unsymmetrical dimethylhydrazine
ON-NCS	Nitrosyl thiocyanate ion	UV	Ultraviolet radiation
c.d.	Outside diameter	wt	Weight
pg	Picogram	yr	Year
pH	Log of the reciprocal of the hydrogen ion concentration; a measure of acidity	Zn	Zinc
Ph	Phenyl group or radical	Zn ⁰	Metallic zinc

ABSTRACT

This report is a review and evaluation of the current knowledge of N-nitrosamines (NA) in the environment as related to possible deleterious effects on human health and welfare. Sources, distribution, measurement, and control technology for NA and their precursors are also considered. N-nitrosamines (characterized by the N-N=O group) are formed by the reaction of amines with nitrous acid. Nearly 70 percent of all N-nitroso compounds studied have been found to be carcinogenic, with a wide range in potency, in all species of laboratory animals tested via all routes of administration. The experimentally produced carcinogenesis appears to be caused by metabolites rather than by nitrosamines themselves. Epidemiological studies to date do not show a direct relationship between exposure to NA and cancer in man.

Nitrosamines can exist in the gaseous, liquid, or solid state, depending on molecular weight. They can be emitted directly to the atmosphere or can enter the environment as a solute in water or hydrocarbons. Because they are rapidly photolyzed by UV light and are rapidly metabolized in animals, no accumulation of nitrosamines is expected to occur in the atmosphere or in the human body.

Ambient air concentrations of NA of up to $36 \mu\text{g}/\text{m}^3$ have been found near an emission source; and of up to $0.2 \mu\text{g}/\text{m}^3$ in major population centers. The relative contributions of natural and man-made sources are indeterminable at present. Average dietary intake of NA is not likely to exceed a few $\mu\text{g}/\text{day}$; intake of NA in municipal drinking water would probably be much less than $1 \mu\text{g}/\text{day}$. Nitrosamines in cigarettes range from 0 to 180 ng/cigarette.

SCIENTIFIC AND TECHNICAL ASSESSMENT REPORT ON NITROSAMINES

I. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

1.1 SUMMARY

1.1.1 Effects

In all species tested in laboratory animal studies, approximately 70 percent of all N-nitroso compounds studied were found to be carcinogenic, with a wide range of potency. There is no direct evidence, however, that nitrosamines have caused cancer in humans, although a few cases of accidental, or occupational, exposure to dimethylnitrosamine are known to have resulted in liver damage. Nitroso compounds have been cited as a possible cause of high rates of cancer in rubber workers, but these workers are also exposed to other chemicals shown to produce cancer in animals. Metabolic studies *in vitro* using rat and human liver slices have shown that the pathways of biochemical changes in the two are similar to some degree. Epidemiological studies to date do not, however, show a direct relationship between exposure to nitroso compounds and cancer in man. It can be concluded, then, that in the few studies in which N-nitrosamines are cited as a possible cancer link the association is implied rather than tested. Thus, no causal relationship between human cancer and nitroso compounds has been established; however, there is no reason to assume that the human species should be immune in view of the available data from animal studies.

The toxic and carcinogenic properties of nitrosamines in experimental animals are well established, with the N-nitrosamines considered to be among the most potent and versatile of all chemical carcinogenic agents.

In laboratory studies, these compounds have produced cancer in over 20 species tested via all routes of exposure in essentially all vital organs. One compound, diethylnitrosamine, has exhibited a toxic effect in rat, mouse, hamster, guinea pig, pig, rabbit, dog, rainbow trout, aquarium fish, grass parakeet, and monkey. The nature of the toxic response apparently is related to the chemical characteristics of the compound administered. The site of activity appears to depend on the compound, the diet, age of animal, species, dosage level, route of administration, and rate of exposure.

Inhalation studies are limited; however, in those conducted to date, the production of tumors of the nasal cavity and other neoplasms in the experimental animals has been reported. In an inhalation study in which rats and mice were exposed to $5\mu\text{g DMN}/\text{m}^3$ for a time equivalent to greater than two-thirds of the lifespan, there was no increase in the incidence of tumors in experimental as opposed to control animals. Oral administration is an effective method of dosage, with small daily doses (a few mg/kg of body weight) over an extended period being more effective than large single doses in producing tumors. Tumors have been induced, though, with a single dose of nitrosamine.

The level of dose required to induce tumors increases with the length of the carbon chain of the alkyl group (27 to 40 mg/kg body weight for dimethylnitrosamine to 1200 mg/kg body weight for dibutyl-nitrosamine). There appears to be a causal relationship between the level of oral dose administered daily and the time of induction or appearance of tumors. At very low doses, the time required for induction might not fall within the normal life span of the animal.

No experimental data are available on the effects in domestic animals of exposure to nitrosamines or their precursors. Nitrites, which are precursors, are formed in the gastrointestinal tract of animals and may be absorbed into the blood, producing methemoglobinemia, especially in ruminants and horses. Nevertheless, no evidence of cancer in domestic animals from the *in vivo* formation of nitrosamines from this and other precursors has been reported.

1.1.2 Physical Properties, Chemistry, and Metabolites

The simple aliphatic nitrosamines are yellow or yellow-green non-hygroscopic liquids that boil without decomposition at 150° to 200°C. The compounds are partially soluble in organic solvents, with solubility in water varying inversely with molecular weight. The simple aromatic nitrosamines are low-melting solids or yellowish oils that are insoluble in water and undergo decomposition at atmospheric pressure. The density of nitrosamines ranges from 0.9 to 1.2 g/cm³, increasing with molecular weight.

The chemistry of nitrosamines relative to the problem of human exposure and biological effects is not well known. It is known, however, that nitrosamines are rapidly photolyzed in the presence of ultraviolet light and are rapidly metabolized in animals. Therefore, one would not expect a persistent buildup of this specific compound in the atmosphere or in the human body. Various studies have been made to assess the potential for the *in vivo* formation of nitrosamines, but the results are not yet conclusive. A large number of variables are involved in the biochemical processes, many of which have not been determined quantitatively in a biochemical system representative of the human environment. Nitrosation in the systems studied has been found to depend on the concentration of nitrite and secondary amines, the pH of the medium, the presence or absence of promoters or inhibitors (ascorbate has been shown to be an effective inhibitor), temperature, time of reaction, the basicity of the amines, and the presence of nitrosating organisms (nitrosation may occur in the intestine or in an infected urinary tract). The possibility of nitrosamine formation *in vivo* resulting from the inhalation of oxides of nitrogen (NO₂) and/or nitrates and amines has not been thoroughly studied. In one study with rats, nitrosamines were found when a gas mixture containing 15 percent NO₂ was bubbled through lung homogenate; but no nitroso compounds were found in lung tissue when the animals inhaled an atmosphere containing 0.01 to 0.5 mg NO₂ per liter.

Early laboratory work demonstrated that nitrosamines are rapidly metabolized, so that even very large doses are removed within 24 hours. Further, the evidence indicates that dimethylnitrosamine is not accumulated in any one organ of the body. The general consensus is that the related carcinogenesis is caused by some active metabolite rather than by the nitrosamine itself. Alkylating agents, such as diazomethane or a carbonium ion, have been suggested as possible active metabolic products of nitrosamines; other suggested intermediates include the corresponding aldehyde, nitrous acid, a hydroxylamine derivative, and a hydrazine derivative (all known mutagens).

1.1.3 Concentrations and Human Exposure

The relative risk of nitroso-compound-induced cancer in humans can be discussed only in a qualitative sense, based on hypothetical reasoning. Given a reasonable qualitative estimate of relative exposure, it is still not possible to translate this to an effective biological burden; i.e., the dose-response relationship at the concentration to which the human population is exposed is not known. If relative exposure is defined simply in terms of total dose over time, then it might be divided into two components: (1) direct exposure to nitrosamines via inhalation, ingestion, and smoking; and (2) exposure to precursors (nitrogen compounds, including amines) via inhalation, ingestion, and smoking.

There are no reliable estimates of the average daily dietary intake of nitrosamines; however, based upon the available measurements, it is not likely to exceed a few micrograms per day. Various nitroso compounds, including N-dimethylnitrosamine, have been identified in a variety of vegetables, fruits, and meats. The concentrations reported vary widely (from non-detectable to mg/kg); however, the validity of the analytical methodology used in many cases is now under question. The long-established practice of using nitrates or nitrites for the curing and processing of meats, and for the preservation of *Clostridium botulinum* (the organism responsible for botulism), may contribute to the amine levels in these products; again, the concentrations vary widely and results are not always consistent. Further, the available information indicates that nitrite levels are being reduced in these food products.

The direct intake from drinking water from municipal water supplies probably would be much less than 1 μg per day. At present there are very limited data regarding the presence of nitrosamines in water, and results to date are not conclusive. Recent studies indicate the presence of N-nitroso derivatives of pesticides in some water samples. Analysis for nitrosamines in a few well-water supplies in the United States, characterized by high nitrate levels and coliform counts, revealed results of ≤ 15 pg/g. Nitroso compounds, as well as nitrates and amines, are found in a variety of pharmaceutical products, constituting another potential for human exposure. Reported values of nitroso compounds in cigarettes range from 0 to 180 ng/cigarette. A one-pack-per-day smoker may then be exposed to a few micrograms per day. Nitrosamines in ambient air at levels up to $36 \mu\text{g}/\text{m}^3$ have been reported near an emission source, while concentrations as high as $0.2 \mu\text{g}/\text{m}^3$ have been reported in major population centers. Assuming 20 m^3 of air inhaled per day, exposure via inhalation again might be a few micrograms per day. It then seems reasonable to assume that direct exposure to nitrosamines via each of the primary routes may be of the same order of magnitude.

The second component of exposure, i.e., the exposure to precursors (nitrogen compounds, including amines) again may be via inhalation, ingestion, and smoking. In this case, the daily intake of both nitrates and amines via ingestion of food and water may be on the order of a few hundred milligrams per day. Nitrosamine precursors are commonly found in foodstuffs and water at much higher concentrations than the nitroso compounds themselves. Nitrite formation in human saliva, particularly among smokers, has been estimated at about 6 to 10 mg/day. Human saliva in smokers also has been found to contain thiocyanate ion, which has been shown to catalyze the reaction of nitrite and secondary amines to form nitrosamines. Tobacco smoke contains several secondary amines and oxides of nitrogen. Nitrate intake via inhalation (ambient air) may be on the order of several hundred micrograms per day in some areas, such as near point sources of emissions. Data are not available on amine intake. Although the reaction between ingested amines and nitrites appears to produce nitrosamines in the digestive tract of animals, almost no studies have assessed the results of inhaling potential nitrosamine precursors. Since the reaction has been found to be enhanced in acidic media, it might be hypothesized that nitrosamine formation in the neutral environment of the lung would be less efficient than under the acidic conditions found in the stomach. Furthermore, preliminary estimates suggest that exposure to precursor amines and nitrogen oxides from food, water, and smoking is probably greater than that through inhalation. Thus, until further evidence is available, risks from inhaled nitrosamine precursors can be viewed as theoretically possible but likely to be of lower order than those from direct nitrosamine inhalation.

Concentrations of nitrosamines in the nanogram to microgram per unit volume or mass (cubic meter, liter, or gram) range now have been measured in air, water, soil, plants, and foodstuff. The environmental loading may consist of both natural and man-made components. Available data do not permit a reasonable estimate of the relative contributions from natural and man-made sources. Further, data are not sufficient to describe the distribution of concentrations in time and space.

If one considers the potential for exposure to nitrosamines or their precursors, then it must be concluded that the entire human population is subject to exposure. Direct exposure to nitrosamines is probably greatest among certain occupational personnel and populations living in the vicinity of primary nitrosamine emission sources, because of additional exposure via inhalation.

The most important gap in our present data involves the biological response in the human population in relation to the potential exposure via all routes. Except for possible occupational or accidental

exposure, the relatively equivalent human exposure is well below the levels used in animal experiments. The problem lies in determining the extent of the environmental hazard and some measure of risk/benefit. This will require a well-organized research effort over an extended period of time. Recommendations for such a research program are outlined in Section 1.3.

1.1.4 Measurement Technology

Technology with which to detect and measure nitrosamines has been developed primarily for the assessment of food and biological samples. Many analytical procedures have been devised. Recently, an improved method has been developed that involves gas chromatography followed by mass spectrometry and a catalytic chemiluminescence technique; the method has sensitivity in the ppt (pg/g) range for food and biosamples. In view of this improved capability, many of the earlier findings of nitrosamines in food are considered questionable. The recent development represents a significant improvement in analytical capability; however, the instrumentation is still in the developmental phase. A standardized monitoring technique suitable for wide field use is still not available.

1.1.5 Sources and Uses

N-nitrosodimethylamine has been used in the past as an industrial solvent (a use now abandoned) and is currently used in one facility as an intermediate in the synthesis of the rocket fuel, 1,1-dimethylhydrazine. Patents have been issued covering the potential use of various nitrosamines as solvents in the fiber and plastic industry; antioxidants in fuels; additions to fertilizers; softeners for copolymers; synergists to self-extinguishing agents used in expanded polymer materials; insect repellants; insecticides, fungicides, bactericides; and lubricating oils.

The major industries involved in the manufacture or use of nitrosamines are rubber processing and the manufacture of organic chemicals and rocket fuel. Several nitroso compounds are available commercially, primarily for sale to the scientific community for research. Some are produced on a large scale, but annual production figures are not available. Diphenylnitrosamine (which to date has not been found to be carcinogenic) is used as a vulcanizing retarder, and dinitrosopentamethylenetetramine is used as a blowing agent in the production of microcellular rubber. One of the main liquid rocket fuels has been 1,1-dimethylhydrazine. A high-yield procedure for preparing unsymmetrical hydrazines involves reduction of the corresponding nitrosamine intermediate.

A wide range of possibilities exists either for the direct emission of nitrosamines into the environment or for the emission of precursors that may result in the formation of nitroso compounds in the environment. Since detailed emission inventories have not been prepared, it is impossible at this time to provide quantitative estimates of environmental loading.

A number of studies have been conducted to determine the emission of nitrosamines from automobiles. Using the best analytical techniques available presently, neither amines nor nitrosamines have been detected in automobile exhaust. Spiking studies strongly indicate that if such compounds are formed in combustion they are destroyed by the exhaust environment. Automobile exhaust samples in light-proof bags were spiked with nitrosamines at levels several times the detection limit. After 30 minutes, less than 30 percent of the original amounts remained.

Application of analytical procedures developed for the determination in ambient air of N-nitrosodimethylamine indicates that this compound is not present in motor vehicle exhaust at the 30 parts per trillion by volume level.

Nitrosamines, if present at all, would be further diluted by ambient air by a factor of at least 100 near roadways and at least 1,000 off roadways. Therefore, it is estimated that auto exhaust contributes either no nitrosamines to the environment, or that potential ambient air levels from such contributions range from below 30 parts per hundred trillion on roadways.

Theoretical considerations and limited chamber experiments indicate that nitrosamines may be formed (primarily during hours of darkness) in a polluted atmosphere containing secondary amines and nitrous acid. The same evidence indicates that the concentrations resulting from such formation should decrease rapidly toward zero soon after sunrise. Available ambient measurements do not

fully support this concept. Logically, it might be assumed that concentrations observed in the ambient air during daylight would be a consequence of direct emissions of nitrosamines from man-made sources; emissions of secondary amines in the presence of sufficient nitrogen oxides concentrations (formation rate would need to exceed destruction rate in sunlight); or direct emissions of nitrosamines from soil or water. These possibilities have not been confirmed. In any event, it is not likely that concentrations in the ambient air would exceed a fraction of a part per billion except very near sources of direct emissions.

Laboratory studies have shown that nitroso compounds can be formed in soil, water, and sewage. Based on these studies, it appears that formation may take place at neutral pH in the presence of colloidal material. Recent laboratory studies indicate that nitrosamines may move rapidly through the soil by leaching into ground water or being taken up directly by plants. Sources from which nitrosamines may be leached include feedlots, which are known to produce a variety of secondary amines at concentrations well above the present detectable level (ppt).

Amines and nitrates which are readily converted to nitrite by microbial actions are ubiquitous in the environment from both natural and man-made sources. Nitrosamines are synthesized by a combination reaction of nitrite or nitrous acid with secondary amines. Hence the necessary precursors for formation of nitroso compounds are ever present.

1.1.6 Control Technology

Control technology has not been adequately developed for nitrosamines. Nitrosamines as a class of compounds can exist in the gaseous, liquid, or solid state, depending on molecular weight. They can be emitted to the environment in any of the phases, as well as enter the environment as a solute in water or hydrocarbons. Available technologies, such as wet scrubbing, incineration, catalytic reduction, and ultraviolet irradiation, should be reasonably efficient in controlling emissions to the atmosphere. Only limited data are available regarding the control of nitrosamines in water (including industrial waste water) and other waste. Again, a wide range of technologies exists from which to select techniques that singly or in combination might prove to be practical for each particular case.

1.2 CONCLUSIONS

In spite of the wide gaps in knowledge of nitroso compounds in the environment and of their impact on human health, a few conclusions appear valid at this time:

- N-nitroso compounds or their metabolites are carcinogenic and mutagenic in a wide range of animal species. Tumors can be induced in essentially all vital organs. N-nitroso compounds are one of the most potent groups of carcinogens, but potency varies over a wide range and depends upon chemical characteristics. These compounds are carcinogenic in experimental animals via all routes of administration and generally act systemically, although some have produced tumors at the site of injection. The tumors induced depend on the route of administration, level of dose, and frequency and length of treatment. Optimum effectiveness in tumor induction appears to be in small doses given over long periods of time. Administration to experimental animals by inhalation has produced tumors in the nasal cavity and in other organs.
- There is no direct evidence that nitrosamines have produced cancer in humans.
- Nitroso compounds are readily metabolized by animals.
- Nitroso compounds can be formed by the reaction of secondary, and some tertiary, amines and nitrites. The reaction, under suitable conditions, may occur in air, water, soil, food, animal stomach or intestines, or in the urinary tract (where it occurs through microbial action).

- Nitroso compounds, or their precursors, are widespread in all media of the environment as the result of contributions of both natural and man-made sources.
- The human population may be continuously exposed to nitroso compounds or their precursors. The consequence of this exposure in terms of human cancer is unknown.
- Although detailed emission inventories have not been prepared, it appears that man-made sources contribute to the environmental loading of nitroso compounds and their precursors and hence may pose a potential health hazard. The extent of man-made contributions is unknown; prudence dictates that adequate control be exercised consistent with economic and social constraints.

1.3 RECOMMENDATIONS

In assessing the need for regulatory or control action relative to environmental pollutants, it is apparent that the research approach in most cases has failed to provide essential information relative to realistic exposure patterns. This is particularly true with toxic or carcinogenic compounds that may be present in very low concentrations from both natural and man-made sources; nitrosamines are such compounds. Since the impact of realistic exposure conditions upon human health or welfare has not been evaluated, the tendency is to adopt the unrealistic position that the exposure level should always be zero. While animal toxicological research provides an excellent basis for identifying potential problem areas, it has not provided effects data at realistic doses. The primary objective in animal experiments has been to obtain positive results, so that doses have not been representative of environmental exposure levels. Occupational studies, when available, provide realistic data for occupational environments but still do not provide definitive information needed for assessing community exposure problems. Consistent results from community studies over long periods of time can provide a valuable body of data, if adequate exposure assessment and experimental design are carefully applied. From a regulatory and control point of view, the research approach should be oriented toward determining exposure conditions that do not produce significant adverse effects. The initial objective would be to establish a level at which observed biological effects are vanishingly small statistically. It would then be necessary to describe a realistic total environmental exposure pattern and compare this to the observed adverse-effects level. Conditions under which the exposure pattern equals or exceeds the no-observed-effects level, in time and space, would be identified. Control strategies and technology would then be developed to handle problem areas. A recommended research approach is described below.

1.3.1 Biological Studies

Biological studies should be designed specifically to determine the dose-response relationship that can be related to current human exposure levels. The program should consist of two phases: (1) direct exposure to nitrosamines; and (2) exposure to precursors that might result in the formation of nitrosamines in the body, both in the respiratory system and the gastrointestinal tract.

1.3.1.1 Lifetime Animal Nitrosamine Exposure—The primary objective is to determine a no-observed-effects level for direct exposure to nitrosamines via inhalation and ingestion. Specific outputs should include: (1) identification of active metabolites and their uniqueness to nitrosamine metabolism; (2) mean distribution and standard deviation of the dose-response curve at low dosages; (3) a time-effect relationship model that might be used for simulation studies and the prediction of cancer in humans resulting from nitrosamine exposure; and (4) the biological effects of nitrosamines in the presence of suspected co-carcinogens or precarcinogens. This program will require a repository of standard reference agents.

1.3.1.2 Nitrosamine Precursor Exposure—The basic objective is to determine the significance of *in vivo* formation of nitrosamines in humans resulting from exposure via inhalation and ingestion to

nitrogen compounds and amines at levels normally found in the environment, and in the presence or absence of inhibitors or promoters.

No data are available on the possible formation of nitrosamines in the lung from the inhalation of precursors. The likelihood of nitrosamine formation in the lung from nitrites and amines is considerably reduced by two facts: (1) the pH of the lung is not normally favorable to nitrosamine formation from these precursors; and (2) nitrite readily combines with the hemoglobin of the blood, and, in a highly vascularized organ such as the lung, would probably be unavailable as a reactant in nitrosation. The possibility of nitrosamine formation in the lung from precursors other than nitrites, in reactions not involving nitrite intermediates, is yet unknown. If it is possible for precursors in the ambient air, such as NO₂ and nitrates, to be transformed to nitrosamines in the respiratory tract, then exposure via inhalation becomes a greater problem. Specific questions that should be studied include:

- Is the respiratory tract capable of reducing inhaled nitrates to nitrite?
- Does the respiratory tract provide the other necessary precursors for the formation of nitrosamines in the respiratory tract?
- What are the various metabolic phases of nitrosamines *in vivo*?
- What enzymes are necessary for this metabolism to occur and are all these enzymes found in man?
- Do salivary nitrite levels vary with differences in exposure to NO₂ and nitrates in ambient air, i.e., to what extent does inhalation of NO₂ and nitrates contribute to nitrite levels found in saliva?
- To what extent does ingestion of precursors in food and water contribute to *in vivo* formation of nitrosamines?

1.3.2 Studies of Population Exposure Patterns

There is a need for much more definitive information concerning population exposure patterns via all exposure routes. Actual exposure will depend on: (1) nitrosamine (and precursor) concentrations in food, water, air (indoor and outdoor), and tobacco; (2) diet; (3) smoking habits; and (4) activity patterns. These studies will require as a prerequisite adequate measurement capability and environmental monitoring for the specific population under study.

1.3.3 Epidemiological Studies

In the final analysis, evaluation of the potential threat to human health due to nitrosamines in the environment will require epidemiological studies. The nature of the chemical carcinogen problem makes it extremely difficult to find exposure patterns that permit the separation of effects of nitrosamines in the presence of a variety of chemicals. Both occupational and community studies will be required. Health indicators should be developed to provide intermediate as well as end-point information. The final output should be a risk/benefit model.

1.3.4 Studies on Environmental Distribution, Transformation, and Removal of Nitroso Compounds

The primary objective is to determine the extent to which nitrosamine and other nitroso compounds may be formed, transported, and accumulated in the environment. The question of the possible formation of nitrosamine in polluted atmospheres should be resolved, as well as the possible accumulation in water supplies.

The studies should cover other similar compounds such as pesticide derivatives. Atrazine, for example, is a principal herbicide used in the U.S. (approximately 90 million pounds used per year). This compound contains two secondary amine groups per molecule and could theoretically form nitroso derivatives from nitrites. A research study should be established to determine, for example,

whether or not nitrosatrazine derivatives are present in surface waters (particularly the Mississippi/Missouri River System) and their persistence and fate in water purification plants. This study should include other nitroso derivatives and should cover such possible sources as sewage treatment plants, feedlots, and certain industries.

1.3.5 Studies on Control Technology

Control methodology for nitrosamines may be approached from two directions, i.e., control of the compounds as they are emitted from chemical processing, or control of the precursors.

Dimethylnitrosamine is the principal nitrosamine of current concern. Water scrubbing of dimethyl-nitrosamine, followed by proper incineration techniques, can effectively limit its emission from industrial sources to the atmosphere.

Amine emissions can be controlled by one or more different techniques that depend on the source of the emission. Methodology employed will vary according to the emission source: manufacture, industrial use, refining, incineration, combustion, and sewage treatment. The use of only limited controls for NO_x and other nitrogenous compounds is possible, however, because these controls are not absolute, and they will probably not have as great an impact as will the control of amines. Although limited controls are presently available, there is still an ongoing research effort to develop catalysts for the control of NO_x emissions from the automobile.

Commitment of resources to the development of technology for controlling higher-molecular-weight nitrosamines or nitrosamine precursors appears questionable at this time for the following reasons:

- Secondary and tertiary amines may react with nitrites in the gastrointestinal (G.I.) tract to form nitrosamines. It is almost impossible to eliminate the ingestion of amines because the amine functional group occurs in drugs, foods, and pollutants. The only possibility of preventing the formation of nitrosamine in the G.I. tract would be to eliminate the intake of NO_x , nitrate, and nitrite.
- The cost of developing and implementing control technology for all possible sources of nitrosamines and their precursors would be quite high.
- Until target emission levels which are based on good dose-response health effects data can be specified, the cost-benefit relationship cannot be established.

2. INTRODUCTION

Nitrosamines have been reported recently in the ambient air in the vicinity of probable sources and in a limited number of water samples. This summary report was prepared to assess the impact of these findings on human health and welfare. The document is not an exhaustive scientific review, with complete bibliography, but a summary of our current knowledge on nitrosamines in the environment that can serve as a basis for regulatory and control decisions. There is an extensive body of literature on the biological activity of nitroso compounds used in laboratory animal studies, but an extremely limited amount of data on these compounds in the air and water. We present information for making prudent decisions on the regulatory issues involved and identify the areas where research is urgently needed.

The concern for nitrosamines in the environment is based primarily upon possible human health effects; therefore, this subject is treated first in the document. For those readers not familiar with the chemistry of nitrosamines, it may be helpful to begin with Section 4.

3. EFFECTS

3.1 EPIDEMIOLOGY

Although biochemical studies have shown that human tissues can metabolize nitrosamines into an active ultimate carcinogen,¹ there is very little evidence that exposure to dimethylnitrosamine, or similar nitrosamines carcinogenic in animals, has led to cancer in man. The epidemiologic evidence for the association of nitrosamines with human cancer is very limited. In the few studies in which nitrosamines are cited as a possible link to the observed cancers, the association is implied rather than tested.

Weisburger and Raineri² have postulated that the appreciable decline in gastric cancer in the United States in the last 40 years may be due, in part, to a better means of preserving foodstuffs at low temperatures. It is believed that during the earlier practice of storing leftover foods at room temperature the nitrate present in the food was reduced to nitrite, and that the nitrite then reacted with naturally occurring substrates such as methylguanidine to form a nitroso derivative which may be carcinogenic. The authors support their argument by pointing out a high correlation between gastric cancers in Chile, Colombia, Eastern Europe, England, and Japan and exposure to environmental nitrates.³⁻⁵

Possible factors contributing to high clusters of cancer of the esophagus in certain sections of Africa have been investigated.⁶ Ingestion of an alcoholic beverage known to contain detectable levels of dimethylnitrosamine was once thought to be a major factor in these high esophageal cancer rates. However, subsequent analysis of the suspect alcoholic samples, based on mass spectrometry, showed that none of the samples contained nitrosamines in excess of 0.005 mg/kg. It was thus concluded that nitrosamines probably could not be implicated in the observed cancers.

There is a very high incidence of esophageal cancer, including cancer of the gastric cardia, in certain areas of North China, where in some communities it ranks as the leading cause of death. Large-scale epidemiologic surveys have been conducted in high- and low-incidence areas to identify geographically related etiologic factors.⁷ Food samples in the high-incidence area contained significantly higher levels of diethylnitrosamine, dimethylnitrosamine, and methylphenylnitrosamine than those in the low-incidence communities. A fungus, *Geotrichum candidum*, which has been shown in the laboratory to act as a co-carcinogen with nitrosamines, was also found in samples of pickled vegetables used as a common food in the high-incidence areas. Although these North China studies strongly suggest a relationship between ingestion of food containing nitrosamines and a fungus which may serve as a co-carcinogen with cancer of the esophagus, the data are limited by the absence of a case-control study. Analyses of gastric juice of noncancerous patients in the high-incidence communities showed the presence of nitrosamines and their precursor compounds, viz., secondary amines, nitrites, and nitrates.

A study of the high incidence of uterine cervical cancer in certain regions of South Africa has revealed the presence of dimethylnitrosamine in discharges from the vaginal vault of pregnant women living in the region.⁸

Exposure of rubber workers to an array of nitroso compounds from both inhalation and skin contact has been cited as a possible cause of the high rates of cancer of the gall bladder, bile ducts, and salivary glands observed in that occupational group.⁹ However, these workers are also exposed to other chemical agents, such as 3,3'-dichlorobenzidine, which are also known to cause similar tumors.

Several cases of accidental dimethylnitrosamine poisoning have been reported.¹⁰ Two men employed by an automotive production facility where dimethylnitrosamine was used as a solvent were accidentally exposed. One of the men recovered after exhibiting signs of liver damage. The other

died and at necropsy a cirrhotic liver with regenerating nodules was revealed. Another report of the hepatotoxicity of dimethylnitrosamine involved three men using the nitrosamine for 10 months as a solvent in a British industrial research laboratory. Two of the three men reported bronchial pneumonia and signs of liver injury, one of which was confirmed as cirrhosis upon necropsy after death. The other technician, who had experienced a hard liver with an irregular surface, recovered after termination of exposure.

Studies by Nicholson et al.¹¹ on various occupational groups exposed to inhalation of nitrosamines thus far have failed to show any association between such exposure and an excess risk of cancer. However, there has been insufficient time since exposure to permit an appropriate latency period.

The lack of strong epidemiologic evidence for an association of human cancer with exposure to nitrosamines may well be a reflection of the limited epidemiologic studies that have been conducted on nitrosamines thus far.

3.2 TOXICOLOGY

3.2.1 Introduction

N-nitroso compounds manifest multipotent biological activity in many species of animals. The biological actions of nitroso compounds include the induction of acute and chronic toxicity, carcinogenicity, mutagenicity, and teratogenicity. Interest in the toxicology of N-nitroso compounds was stimulated when N-nitroso-N-methyl urethane^{12,13} and dimethylnitrosamine^{14,15} were recognized as industrial hazards. In an American automobile factory, where dimethylnitrosamine was used as a solvent, two men were accidentally poisoned. One man recovered after signs of liver damage; the other died in a clinical accident and a necropsy revealed a cirrhotic liver with regenerating nodules.¹⁴ Two of three men in a British industrial research laboratory, working with a solvent over a period of 10 months, showed signs of liver injury. One died of bronchopneumonia and a necropsy showed liver cirrhosis. The other technician developed a hard liver with an irregular surface but recovered after exposure to the solvent was terminated.¹⁵ In characterizing the toxicity of dimethylnitrosamine, Magee and Barnes¹⁶ discovered its carcinogenic action in rats. The toxicology of N-nitroso compounds has been reviewed by Magee and Barnes,¹⁷ Druckrey et al.,¹⁸ Magee,¹⁹ Magee and Swann,²⁰ and, more recently, by Shank.²¹

3.2.2 Acute Toxicity

The potency of N-nitroso compounds in causing acute tissue injury and death varies considerably (Table 3-1). Acute toxicities of N-nitrosamines and N-nitrosamides expressed as single-dose oral LD₅₀ values in adult rats range from a low of about 20 mg/kg to a high of more than 5000 mg/kg, with many compounds having an LD₅₀ between 150 and 500 mg/kg. The more reactive compounds produce hemorrhagic destructive lesions at the site of contact; others result in hepatic damage, pulmonary lesions, and convulsions. Spills have led to irritation of eyes, lungs, and skin. The histopathology of acute poisoning from dimethyl- and diethylnitrosamine has been well studied. Dimethyl and diethylnitrosamine are hepatotoxins causing centrilobular necrosis with hemorrhages in 24 to 48 hours; death occurs in 3 to 4 days or the animals survive and recover completely in approximately 3 weeks. Manifestations of N-nitrosamine hepatotoxicity include "blood cysts" — areas of destruction of the parenchyma filled with recently extravasated erythrocytes — and necrosis of the endothelium of the central and sublobular veins with extrusion of necrotic hepatic parenchymal cells into the lumen. The kidney lesions are limited exclusively to the convoluted renal tubules. Rats treated with sublethal doses of dimethylnitrosamine developed veno-occlusive lesions in the liver.³¹ Veno-occlusive lesions have also been seen in mink treated with dimethylnitrosamine.³² Typical acute liver changes have been induced in rats by the heterocyclic nitrosamines.^{29,33} The N-nitrosamides are extremely irritating locally; for example, N-methyl-N-nitrosourea causes severe necrotic stomach lesions after gavage.¹⁸ N-methyl-N-nitrosourea given systemically has induced acute effects on the bone marrow of hamsters³⁴ and mice.²⁰ N-nitro-

**Table 3-1. ACUTE TOXICITY OF SOME
N- NITROSO COMPOUNDS**

Compound	LD ₅₀ ^a	Reference
Dimethylnitrosamine	27 to 41	22
Diethylnitrosamine	216	22
Di- <i>n</i> -propylnitrosamine	400 ^b	23
Di- <i>n</i> -butylnitrosamine	1200	24
Di- <i>n</i> -amylnitrosamine	1750	25
Methyl- <i>n</i> -butylnitrosamine	130	22
Methyl- <i>t</i> -butylnitrosamine	700	22
Ethyl- <i>n</i> -butylnitrosamine	380	26
Ethyl- <i>t</i> -butylnitrosamine	1600	26
Ethyl-2-hydroxyethylnitrosamine	7500	26
Di-2-hydroxyethylnitrosamine	5000	27
Methylphenylnitrosamine	200	22
Methylbenzylnitrosamine	18	26
Nitrosomorpholine	282	25
Methylnitrosourea	180	25
Methylnitrosourethane	240	28
Nitrosohexamethyleneimine	336	29
Nitrosoheptamethyleneimine	283	30
Nitrosooctamethyleneimine	566	30

^a LD₅₀ units: mg/kg body weight, single oral dose, adult male rats.

^b Male and female Syrian golden hamsters.

samides, such as N-methyl-N-nitrosourea and N-methyl-N-nitrosourethane, have moderate LD₅₀ values.

A great deal of work remains to be done in correlating the structure and the acute toxicity of N-nitrosamines. It does seem, according to Shank,²¹ that acute toxicity decreases with chain length of dialkylnitrosamines. The predominant effect of liver damage caused by dialkylnitrosamines seems to be best explained by the assumption that these compounds require metabolism for their tissue-damaging action and that the adversely active molecular species is a metabolite of the parent nitrosamine. This hypothesis would explain the relatively selective action on the liver since, generally speaking, the enzymes responsible for the metabolism of drugs and other foreign compounds are present in highest amount in the liver. Detailed studies of the acute toxicity of the N-nitroso compounds as a class have not been common because the striking carcinogenicity of many of these compounds has commanded such intense interest.

3.2.3 Carcinogenicity

The N-nitrosamines are highly potent and versatile carcinogens. Since the first report of hepatic cancer induced in rats by dimethylnitrosamine,¹⁶ many more alkylnitrosamines have been found to induce tumors in various species in a wide range of organs. While the induction of cancer in the rat from nitrosamines has been studied most frequently, cancer has been detected also in the mouse, hamster, rabbit, guinea pig, dog, monkey, and fish. Among the organs affected have been the liver (most often), kidney, lung, stomach, esophagus, bladder, nose, forestomach, intestine, and tongue. Since large doses of these compounds result in extensive liver damage and toxic effects, cancer studies have been conducted in most cases by feeding animals daily doses, generally between 5 μ g and 100 μ g, of nitroso compounds. Not only has it been produced by oral administration, but cancer has also been produced by injection, inhalation, and skin application.

The route and time of exposure affect the total dosage of nitrosamine required for development of cancer. The smaller the daily dose, the smaller is the cumulative total dose administered by the time

of appearance of cancer. With smaller doses, the median time to appearance of cancer is lengthened.³⁵ The lowest dose required to produce a carcinogenic response is not known with any degree of certainty. At a dietary concentration of 2 mg DMN/kg of feed, Terracini et al.³⁶ found liver tumors after 104 weeks in 1 of 26 surviving rats. Tumor formation occurred after the same length of time in 8 of 76 when the dietary concentration was 5 mg DMN/kg of feed. The lowest dose at which tumors were observed corresponded to a daily dose of 0.12 mg DMN/kg body weight (B.W.) for an average rat (0.25 kg). In a similar study, Druckrey et al.¹⁸ reported that at the lowest dose level they used, 0.075 mg/kg B.W. per day, 20 animals survived for more than 600 days. Of this group, 11 had benign or malignant tumors of the liver and of the esophagus and all 4 rats that survived more than 940 days developed tumors. This is the lowest level of any nitrosamine yet reported to produce a carcinogenic response. However, an important unknown factor in these ingestion experiments is the possibility of *in vivo* formation of nitrosamines.

A study was initiated by Thomas³⁷ to test the effects of chronic inhalation of unsymmetrical dimethylhydrazine (UMDH). Dimethylnitrosamine (DMN) is an intermediate in the synthesis of UMDH and has been shown to be a contaminant of the UMDH used in this study. Overt signs of toxicity were nonexistent in mice, hamsters, rats, and dogs exposed for 6 months to 6.0 ppb (plus 5.0 ppm UMDH) and 0.6 ppb (plus 0.5 ppm UMDH) N-nitrosodimethylamine, respectively. Significant exposure effects at 6 months were limited to slight-to-moderate hepatotoxicity in dogs exposed to the higher level of UMDH plus N-nitrosodimethylamine, with indications of partial recovery apparent by the end of 4 weeks post-exposure. Hepatotoxicity was measured by the assay of serum glutamic-pyruvic transaminase levels and by bromsulphalein retention.

This study was expanded to include an examination of the dose-response relationship between oral DMN administration and hepatotoxicity.³⁸ Oral doses were determined by calculating them as equivalents of doses received from 6-hour inhalation exposures to DMN; the standard minute volume for 25-g mice was used in the calculations and the assumption was made that absorption of DMN was 100 percent for both routes of exposure. Male mice were dosed with 5, 10, 50, 500, 1000, and 5000 μ g/kg B.W. Mice given the lowest two levels, 5 and 10 μ g/kg B.W., were dosed 5 days/week for 60 calendar days. Mice given the higher levels were dosed for 28 calendar days. Five mice from each group (including a control group) were sacrificed at 7, 9, 14, 21, and 28 days. All survivors of the four higher doses were sacrificed at 28 days. Livers were removed from animals that died or were sacrificed and were examined for gross and histopathology. Each group of mice contained extra animals to compensate for losses from toxicity or from the gastric intubation procedure itself. Of the 327 mice tested, 25 died during the experiment, most of them from pneumonia. Only 1 of the 25 — a mouse receiving the highest dose — died from chemical toxicity; necropsy showed severe, massive liver necrosis.

Grossly, some enlargement of one or more lobes of the liver was seen in all groups except the control group. In addition, mice receiving the highest dose showed subcutaneous edema, ascites, and roughening of the liver capsule. Histological examination showed vacuolization of hepatocytes in all groups (including controls), where it was thought to be an artifact formed during anesthesia prior to sacrifice.

Results of histological examinations are summarized in Table 3-2.

In a recent inhalation study by Moiseyev and Benemanskii,³⁹ mice and rats were subjected to continuous exposure of low levels of dimethylnitrosamine (DMN) for 17 months. Dose levels were 0.2 mg/m³, 0.005 mg/m³, and 0 mg/m³. Significant increases in both benign and malignant tumors were noted for groups exposed to 0.2 mg/m³, with tumors occurring earlier than in the controls and in a variety of organs. Tumors developed in 42 of 51 rats (82 percent) exposed to 0.2 mg/m³ as compared to tumors in 22 of 75 (29 percent) controls. Animals (78 rats and 68 mice) inhaling 0.005 mg/m³ of DMN did not differ from controls. The 0.2 mg/m³ dose level corresponds to 0.170 mg/kg body weight per day for rats. Wistar rats were more susceptible to neoplasm development than Balk/G mice.

Diethylnitrosamine (DEN) has been shown to exhibit an organotropic toxic and carcinogenic activity in the respiratory system, including the nasal cavity of many species of animals.⁴⁰⁻⁴¹ Some of the neoplasms of the nasal cavity of these animals induced by DEN are histologically and biologically similar to those seen in humans.⁴² Inhalation of nitrosamines produced tumors of the upper

**Table 3-2. HISTOPATHOLOGY OF LIVER TISSUE
FROM MICE GIVEN DIMETHYLNITROSAMINE ORALLY³⁸**

Oral dose, μg/kg	Inhalation equivalent (6-hr), ppb	Hepatic response	No. animals with response/ total no. animals	Percent of animals with response
0	0	Vacuolization	40/61	65
5	5	Vacuolization	48/61	78
10	10	Vacuolization	53/59	90
50	50	Vacuolization	38/38	100
500	500	Vacuolization	33/36	91
1000	1000	Vacuolization	35/36	97
5000	5000	Vacuolization and multifocal hepatic necrosis	35/36	97
			34/36	94

respiratory tract of hamsters.⁴³ Neoplasms have also developed in the offspring of female mice and hamsters⁴⁴ and rats⁴⁵ treated during pregnancy with several nitroso compounds. The transfer of DEN through rat milk has been shown.⁴⁶ Lactating rats 5 days post-partum were given 130 mg/kg body weight of DEN by gavage. The stomach contents (mainly curdled milk) of the suckling pups of these rats were analyzed for the presence of DEN and were found to contain 5, 16, and 36 mg/kg DEN at 2, 4, and 6 hours, respectively, after they started ingesting mother's milk. No DEN was detected in the mother's milk 49 hours post-gavage. These concentrations may not reflect exact amounts of DEN transferred through mother's milk, however, as the young might have contained some milk without DEN from previous suckling and, most important, there may be active process(es) of diffusion and digestion of DEN from the stomach of the young. This would mean that each pup probably received 20 μg of DEN within the time that the DEN was present in mother's milk. Suckling young rats that received DEN through mother's milk subsequent to several administrations of DEN (130 mg/kg) to the nursing mothers developed multiple neoplasms.⁴⁷ These observations show that the induction of neoplasms in the offspring may have been due at least in part to the ingestion of unaltered DEN through the mother's milk.

Both nitrosamides and nitrosamines appear to induce neoplasms transplacentally. N-nitrosoethylurea given to pregnant rats on the 15th day of gestation produced brain and spinal cord tumors in the offspring;⁴⁸ exposure during days 10 through 21 of gestation have resulted in renal tumors in the offspring several months after treatment.⁴⁹ Diethylnitrosamine given subcutaneously to pregnant rats on days 9 through 15 of gestation induced tracheal papillomas in almost half the offspring within 25 weeks of the first administration.⁵⁰

Recently, hamsters have been shown to develop neuroepithelial neoplasms (esthesioneuroepitheliomas) of the nasal cavity when dimethylnitrosamine (DMN) was given subcutaneously and ferric oxide (an inert particulate material) installed intratracheally;⁵¹ DMN alone did not induce nasal cavity neoplasms in this experiment or in others. This is an example of modification of the neoplastic expression of a known carcinogen by respirable particulate material. The intragastric administration of methylicholanthrene and the intraperitoneal administration of DMN to Swiss mice resulted in an increased incidence and decreased latency period of neoplasm, compared with the mice treated with either compound.⁵²

Rats have been shown to develop sarcomas at the site of subcutaneous injections of the N-nitroso derivative of the pesticide, carbaryl (N-methyl-1-naphthyl carbamate). In studies of the carcinogenic activity of N-nitrosocarbaryl, 14 of 16 Wistar rats developed tumors at the site of a single subcutaneous dose (1000 mg/kg B.W.) and died within 450 days after injection. No other tumors or toxic effects were found. In 37 Wistar rats given a single oral dose of N-nitrosocarbaryl (200 to 1500 mg/kg B.W.), no tumors or other toxic effects had occurred at the end of 21 months, or about 638 days.⁵³

Several cyclic nitrosamines induce neoplasms of the liver and other organs.⁵⁴ A high incidence of neoplasms of the liver, tongue, and esophagus was seen in rats given N-nitrosohexamethyleneimine in drinking water.⁵⁹ N-nitrosoheptamethyleneimine (N-6-MI) produced squamous neoplasms of the lung and esophagus in rats.⁶⁰ Subsequently, Lijinsky and his colleagues⁵⁵ induced a high incidence of squamous neoplasms of the lung in rats by feeding heptamethyleneimine together with nitrite. Although some nitroso compounds are equally carcinogenic independent of the route of application, the N-6-MI was shown to be less effective when given subcutaneously.⁵⁶ However, recent results show that a single subcutaneous injection of N-6-MI induced a high incidence of respiratory tract neoplasms in hamsters and mice.⁵⁷ In an attempt to evaluate respiratory infection as a cofactor in the development of respiratory tract neoplasia, germ-free, specific-pathogen-free and infected (chronic murine pneumonia) rats were given N-6-MI in drinking water for 22 weeks. Rats were sacrificed 2 weeks post-treatment. The incidence of lung neoplasms indicated that chronic respiratory infection enhanced the neoplastic response of the lungs to a systemic carcinogen.⁵⁸ These data show that cyclic, non-alkylating nitroso compounds induce neoplasms similar to those induced by most non-cyclic nitrosamines^{43,59} and by alkylating cyclic nitrosamines.⁶⁰ Aminopyrine has been shown to yield dimethylnitrosamine *in vitro*⁶¹ and to induce malignant neoplasms of the liver on continued simultaneous administration with nitrite.⁵⁵ Sprague-Dawley rats given 1 g/kg of solution or 0.25 g/kg of solution of aminopyrine and sodium nitrite for 30 and 50 weeks in drinking water, respectively, developed hemangioendotheliomas of the liver.⁶² The current permissible sodium nitrite level in processed meats in the U.S. is 0.2 g/kg.

The possibility that nitrosamines are formed from nitrite and secondary amines under the acidic conditions of the stomach was hypothesized^{63,64} and shown to be a reality.^{65,66} Recently, lung adenomas were induced in Swiss mice chronically fed nitrite plus morpholine, piperazine, or N-methylaniline.⁶⁷ The continued administration of morpholine or methylbenzylamine simultaneously with nitrite to rats in their drinking water induced the tumors expected from the known carcinogenic effects of the corresponding nitrosamines.⁶⁸ However, no neoplasms were induced in rats by feeding secondary amino acids — proline, hydroxyproline, and arginine — concurrently with nitrite.⁶⁹ An extension of the above hypothesis^{63,64} is to study the formation of nitrosamides from corresponding alkylureas and nitrite under acid conditions. Sander and Burkle⁷⁰ induced a spectrum of neoplasms in rats by feeding nitrite together with N, N'-dimethylurea, methylurea, ethylurea, or 2-imidazolidone (a cyclic urea). Neurogenic neoplasms in the offspring of female rats fed nitrite and ethylurea during pregnancy were seen.⁷¹ These neurogenic neoplasms are similar to those induced in the offspring of rats given nitrosamides during the latter half of pregnancy.⁴⁸ Recently nitrosamide formation *in vivo* has been shown in Swiss mice fed nitrite and methylurea and ethylurea concurrently.⁷²

Druckrey et al.¹⁸ conducted an extensive study of 65 nitroso compounds to show carcinogenic activity and to correlate potency and type of cancer with chemical structure. All symmetrically substituted dialkylnitrosamines produced carcinomas of the liver. The only exception was the dibutyl compound, which on subcutaneous injection also produced bladder cancer. The diamyl derivative resulted selectively in pulmonary cancer. Four other symmetrical nitrosamines proved noncarcinogenic: allyl, cyclohexyl, phenyl, and benzyl. Asymmetrical dialkylnitrosamines, especially when one of the alkyl groups was a methyl group, produced carcinomas of the esophagus; methylallylnitrosamine induced tumors of the kidney. Cyclic nitrosamines, synthesized for example from nitrite and pyrrolidine or morpholine (nitrosopiperidine or nitrosomorpholine), produced cancer of the liver. Nitrosopiperidine also resulted in carcinoma of the esophagus. Nitrosamines with other chemical functional groups in the structure produced malignant tumors as well. Several acylalkylnitrosamines gave carcinoma of the forestomach on oral application but only local tumors at the site if the material was injected. Of the approximately 100 N-nitroso compounds that have been studied, more than 80 have been shown to be carcinogenic in test animals.⁷³

In a study still in progress,⁷⁴ four groups of Sprague-Dawley rats were given N-nitrosopyrrolidine daily in the drinking water "at concentrations ensuring the required dosage." A fifth group served as controls. All animals were carefully observed until they died or became moribund, the rats being sacrificed in the latter case. Of 24 rats treated with 10 mg/kg B.W.-day, all had died by day 600; 3 by day 322 without tumors, 5 with benign tumors, and 10 with malignancies, 9 of which were hepato-

cellular carcinomas. Mean tumor induction time was 436 days and the mean total dose applied was 4033 mg/kg B.W. A second group of 39 rats was treated with 3 mg/kg B.W.-day. Of these, 35 developed tumors, 34 of which were in the liver. Mean induction time was 515 days and the mean total dose was 1442 mg/kg B.W. Doses given to the third and fourth groups, 1.0 and 0.3 mg/kg B.W.-day, respectively, had not led to the induction of typical liver tumors after about 660 days of treatment, with 50 percent of the animals still living at that time. Among the control rats, pneumonia was the main cause of death, though two controls had malignancies and two had benign tumors. The medium life expectancy of the controls was about 550 days.⁷⁴

In the same study, the nitroso derivatives of two pesticides were administered to rats by stomach tube. Two groups of rats were given aqueous solutions containing 20 mg/kg and 5 mg/kg, respectively, of N-nitrosobenzthiazuron once each week. At the higher dose, 51 of 62 rats developed tumors, with a mean induction time of 448 days and a mean total dose of 1156 mg/kg B.W. The tumors were localized mainly in the forestomach (31), kidney (17), and liver (11). At the lower dose, 24 of 64 rats were still living at the end of 600 days of treatment. Seven of the 40 animals that died before day 600 had tumors, including one each in the liver and the forestomach.

The second nitrosated pesticide given was N-nitrosocarbaryl, which was administered in a vegetable oil suspension by stomach tube in doses of 130 mg/kg B.W. twice weekly. Mean tumor induction time was 167 days, with tumors induced in 17 of 32 rats. The forestomach was again the target organ.

Table 3-3 lists some of the N-nitroso compounds and some sites where they induce tumors.

Table 3-3. SITES OF TUMORS PRODUCED BY SOME N-NITROSO COMPOUNDS

Site	Compound	Reference
Skin	Methylnitrosourea	76
Nose	Diethylnitrosamine	77
Nasal sinus	Dimethylnitrosamine	78
Tongue	Nitrosohexamethyleneimine	29
Esophagus	Nitrosoheptamethyleneimine	30
Stomach	Ethylbutylnitrosamine	17
Duodenum	Methylnitrosourea	79
Colon	Cycasin	80
Lung	Diethylnitrosamine	44
Bronchi	Diethylnitrosamine	44
Liver	Dimethylnitrosamine	16
Pancreas	Nitrosomethylurethane	81
Kidney	Dimethylnitrosamine	82
Urinary bladder	Dibutylnitrosamine	83
Brain	Methylnitrosourea	84
Spinal cord	Nitrosotrimethylurea	85
Thymus	Nitrosobutylurea	86
Lymph nodes	Ethylnitrosourea	87
Blood vessels	Nitrosomorpholine	88
		89

There is good correlation between metabolism of nitrosamines and tumor induction.⁷⁵ Dimethylnitrosamine is readily metabolized by rat liver, less so by rat kidney and lung; liver tumors are the most frequent neoplasms obtained with dimethylnitrosamine administration; renal tumors are much less frequent; and lung tumors are seen only under special experimental conditions.¹⁷ Diethylnitrosamine is readily metabolized by hamster lung but much less so by hamster liver, and its administration to hamsters results in more lung tumors than liver tumors. The nitrosamides, presumably

because of their instability in neutral and alkaline solutions, produce tumors at the site of administration or where decomposition is favored.

3.2.4 Mutagenicity and Teratogenicity

Many carcinogenic nitroso compounds are mutagenic,¹⁷ and a few have been found to be teratogenic.

The nitrosamides, but not the nitrosamines, are mutagenic in *in vitro* bacterial systems. The nitrosamines appear to require metabolic activation by mammalian enzyme systems before they can exert a mutagenic effect. This was demonstrated by Popper et al.,⁹⁰ who added dimethylnitrosamine (DMN) directly to cultures of the *Bacillus subtilis* 168 *ilv* auxotroph and found that DMN by itself was not mutagenic at concentrations as high as 300 mM. DMN became mutagenic for this auxotroph when DMN and the bacteria were incubated with mouse liver microsomes in the presence of an NADPH-generating system. Czygan et al.⁹¹ subsequently found that the oxidative demethylation of DMN and its transformation to a mutagen are both dependent on the cytochrome P-450 content of microsomes. Cytochrome P-450 is the terminal oxidase in the microsomal biotransformation system that metabolizes steroids, drugs, and carcinogens and other foreign compounds.

In a subsequent experiment, Czygan et al.⁹² studied the capacity of human liver microsomes (from patients undergoing abdominal surgery) to alter the mutagenicity of DMN. They found in humans, as in mice, that DMN mutagenicity was activated by liver microsomes in the presence of an NADPH-generating system and that the resulting mutagenicity was proportional to the cytochrome P-450 content of the microsomes. In the livers of the 29 patients examined, the microsomal cytochrome P-450 varied over a twelvefold range. The microsomes showed a proportional differential ability to alter the mutagenicity of DMN in the *in vitro* assay system. Czygan et al. stated that the observed variations in cytochrome P-450 content in the livers "may be related to diseases, therapy, or environmental pollutants."

Czygan et al.⁹³ also investigated the effects of dietary protein and choline levels on the ability of isolated rat hepatic microsomes to alter the mutagenicity of DMN. They found a positive correlation between dietary protein and choline content and microsomal protein and cytochrome P-450 content. They concluded that the status of the microsomal biotransformation system that activates the mutagenicity of DMN can be influenced by nutritional factors.

Chromosomal changes have been obtained with sodium nitrite by Fahmy et al.,⁹⁴ who suggested that more than one mechanism could be at work in mutagenicity, depending on pH: action by nitrite at low pH or by an alkylating agent at higher pH. It was further pointed out that metabolically produced aldehydes and their reduction products, such as hydroxylamine or hydrazines, could be the active mutagens since such compounds are mutagenic in some systems.⁹⁴

N-nitroso compounds have also been shown to be potent teratogens. N-nitrosomethylurea given to rats on the 13th or 14th day of gestation results in fetal deaths and resorption and deformities in those that reach term.⁹⁵ When N-nitrosoethylurea is given to rats before the 12th day of pregnancy, the compound is not carcinogenic but is a powerful teratogen.⁹⁶

3.2.5 Mechanism of Action

The mechanism of acute toxicity and carcinogenesis has been the object of a great deal of research. A few basic features are well agreed on: nitrosamides are easily decomposed to products that apparently damage any cells that they contact, hence give rise to cancer at the site of exposure. Nitrosamines are more stable, require enzymatic action, and are therefore active only in organs that provide the proper enzyme system. The active intermediate agent, the proximate carcinogen, can be one of several chemical species, including nitrite, hydroxylamine, a hydrazine derivative, or an alkylating intermediate, possibly a diazoalkane or carbonium ion. Alkylation of the 7 position of guanine in nucleic acids, which has been observed in somatic organs and which may later produce tumors, is essentially the same as that which occurs with DNA of germ cells and is thought to be responsible for genetic mutations. Methylation of messenger RNA resulting from dimethylnitrosamine poisoning effectively inhibits translation in protein synthesis^{97,98} by inactivating guanine in the genetic code.⁹⁹ Ludlum¹⁰⁰ has shown, however, that polyribonucleotides containing 7-methyl-

guanylic acid serve as normal templates for RNA polymerase. Incorporation of 3-methylcytosine in polycytidylic acid, however, not only lowered template efficiency in producing polyguanylic acid, but also produced incorrect polymers of guanylic and uridylic acids. This suggests that alkylation of cytosine at the 3 position may be more closely related to carcinogenesis than alkylation of the 7 position of guanine.

Some maintain that denaturation of cellular macromolecules involved in metabolic control is an alternative path of carcinogenesis.¹⁰¹ This idea is partly based on shortcomings of the alkylation hypothesis. For example, the heterocyclic nitrosomorpholine cannot form a diazoalkane or other alkylating intermediate (there is no experimental proof that the ring can be opened metabolically), yet it is carcinogenic. Similarly, phenylmethylnitrosamine, which has caused cancer of the esophagus in the rat, does not form methyldiazonium ion.

Furthermore, there are a number of instances in which there is poor correlation between the formation of 7-methylguanine and a compound's ability to induce tumors in specific tissues. Methylnitrosourea is a potent brain carcinogen and its administration produces approximately the same level of 7-methylguanine in brain DNA as does methylmethanesulfonate, which is only a weak brain carcinogen.^{102,103} Methylnitrosourea treatment produced 8-methylguanine, which was not detected in brain DNA from methylmethanesulfonate-treated rats. Loveless¹⁰⁴ has suggested that this minor base may be more important in mutagenesis and carcinogenesis than 7-methylguanine.

Whatever the initial step in carcinogenesis, it seems plausible that some change in the heritable properties of the transformed cells must occur. The result of alkylation of DNA, the genetic material in most organisms, is thought to involve a change in base sequence or the loss of one or more base pairs.¹⁰⁵

3.3 HUMAN HEALTH HAZARD

A causal relationship of nitrosamines to human cancer is suggested by the following: (1) All mammals studied have been shown to be susceptible to tumor induction by at least one nitrosamine; (2) nitrosamines (or their metabolites) are highly effective carcinogens by any route of administration — inhalation and oral intake included; (3) some of the animal neoplasms induced by nitrosamines are predominantly epithelial and their organotypic and histologic features resemble those seen in humans; (4) nitrosamines are metabolized *in vitro* similarly by human, guinea pig, and rat tissue; (5) nitrosamines occur in the human environment — cured meat and fish products, tobacco and tobacco smoke, and ambient air; and (6) evidence is conclusive that nitrosamines or nitrosamides can be formed *in vivo*, most likely in the mammalian stomach or possibly in the lower gastrointestinal tract, through interaction of secondary and tertiary amines ingested as food or drugs with nitrite and as a result of microbial metabolism.

The cause and effect relationship between certain environmental and pharmaceutical chemicals, and the development of certain human neoplasms, has been shown. The incidence of "cancer" in man is quite high, and the distribution of "cancers" of certain types in some segments of the population seems to reflect well-defined exposures to carcinogens. Although the daily exposure of man to nitrosamines is unknown, nitrosamine precursors are ubiquitous in the environment. Continued, chronic exposure to these nitrosamine precursors could conceivably result in the formation of nitroso compounds; and, even if such nitroso compounds were present at very low levels, unremitting insults from these compounds over several decades might contribute to human cancer. Moreover, the distinct possibility that other noxious or etiologic agents could act synergistically with nitrosamines to modify their biological effects should be considered. It should be noted in this discussion, however, that only a few nitrosatable precursors, and their respective nitrosamine derivatives, have been shown to induce neoplasms in laboratory animals.

3.4 ECOLOGICAL EFFECTS

3.4.1 Plants and Microorganisms

The presence of nitrates, nitrites, or nitrosamines in the environment does not usually present a hazard to plants or microorganisms. Nitrates are an integral part of the life cycle of plants and many microorganisms.¹⁰⁶⁻¹⁰⁹ Nitrates are of great importance in plant nutrition and constitute the chief form of nitrogen in soils available to plants.¹⁰⁶⁻¹¹⁰ As indicated later (Section 7), nitrates are formed, assimilated, and transformed by microorganisms during the nitrogen cycle.

Nitrites in small amounts are not hazardous to plants or microorganisms.^{106-108,110} Nitrites in low concentrations may be taken up by plants, while in high concentrations they are injurious to plant roots.¹¹¹ Plants vary in their sensitivity to nitrites. Legumes are more susceptible than grasses. For example, when 376 kg/ha of sodium nitrite was applied to pasture grasses, no visible damage occurred and corn (*Zea mays*) grown in water cultures was able to tolerate up to 200 mg/liter of nitrite nitrogen.¹¹⁰

No nitrosamines were detectable in spinach containing nitrite.¹¹² It was hypothesized that the weakly alkaline medium in spinach and low concentrations of secondary amines were important factors in limiting the synthesis of nitrosamines even when high nitrite levels were present.¹¹¹

Microorganisms are extremely sensitive to nitrite in high concentrations although nitrite is formed in low concentrations by microbial activity. Nitrite accumulation inhibits soil-borne fungi, such as *Fusarium oxysporium*, as well as many bacteria.¹⁰⁷ Nitrites have also been shown to be mutagenic in some fungi¹¹² and bacteria.¹¹⁰

Microorganisms appear to play an important role in the formation of nitrosamines.¹¹³⁻¹¹⁵ Recent evidence indicates that nitrosamines in soil and water are stable and are not readily degraded by microorganisms present in the environments where they are formed.¹¹³⁻¹¹⁵ Nitrosamines have not been shown to be toxic to microorganisms.¹¹⁶

Plants are capable of assimilating nitrosamines from the soil and from water cultures.^{117,118} They are also capable of forming nitrosamines by metabolic activity.¹¹⁹ The presence of nitrosamines in the leaves, stems, and fruits of plants appears to have no detrimental effect on the plants.¹¹⁸ The nitrosamines absorbed by plant roots do not accumulate to any extent within the plant but appear to be metabolically broken down and to disappear quite rapidly from the plant. Therefore, decreasing concentrations of nitrosamines in the growth media result in a decrease in plant uptake and a rapid decrease in the concentrations in plants due to metabolic breakdown. The mechanism by which plants break down nitrosamines is not known at present. The metabolites formed from nitrosamine breakdown by plants also are not known.¹¹⁸

The nitrosamine, 4-(nitrosomethylamino)-benzaldehyde, was found in a culture medium in which the fungus, *Clitocybe suaveolens*, was grown; however, it is not known whether this is a *de novo* synthesis of the nitrosamine or whether the fungus provided a medium in which the synthetic reaction could take place.

3.4.2 Domestic Animals

Nitrates become hazardous to animals when they accumulate in food plants and are ingested by animals.¹⁰⁶ "Nitrate toxicity" is in reality "nitrite toxicity" and occurs following the reduction of nitrate to nitrite within the gastrointestinal tract of animals.^{106,120-122} Nitrite moves into the blood stream, oxidizes the ferrous iron of hemoglobin to ferric iron, producing methemoglobin, which is incapable of transporting oxygen to body tissues.^{106,121} Methemoglobinemia is the term used to designate this condition.

Horses and ruminants, because of their anatomy and the microflora of their digestive tracts, are most susceptible to the reduction of nitrates to nitrites prior to their absorption into the circulatory system.^{106,121} Monogastric animals are, for the most part, not easily poisoned by nitrates. They are, however, susceptible to nitrites.^{106,121}

Nitrate toxicity may be acute or chronic.^{106,121} Acute toxicity results in death within a few hours. Chronic toxicity, caused by the consumption of low levels of nitrate over a period of time, results in a general debilitation of the animals.^{106,121,122} The debilitation may be the result of vitamin deficiency, for nitrite appears capable of destroying carotene and vitamin A.¹²¹ Thyroxine, a hormone which controls the rate of metabolism and which is produced by the thyroid gland, may be affected by nitrite.¹⁰⁶ Abortion in cattle may be another effect of the chronic ingestion of nitrate.^{106,121,122} Clinical signs associated with chronic nitrate poisoning in animals include dyspnea, grinding of teeth, uneasiness, vasodilation, lowered blood pressure, reduction of milk secretion, abortion, avitaminosis A, and thyroid dysfunction.¹²³

Animals normally susceptible to "nitrate toxicity" can adapt to the intake of higher levels of nitrate than usual.¹¹¹ Experiments in which pregnant heifers were fed 440 to 660 mg of nitrate per kg body weight over a long period showed that the heifers adapted to a deficiency of oxygen in their blood by increasing the number of circulating erythrocytes.¹¹¹ Other experiments have shown cattle and sheep capable of adapting to high levels of nitrate.

For many years, farmers in South Africa have known that ruminants could tolerate higher levels of nitrate when fed molasses. Because of the South African information, livestock feeders are aware of the importance of feeding high-energy feeds when nitrates are present in appreciable amounts. High-energy nutrients are thought to produce a more intense reducing environment in the rumen, so that nitrates are reduced beyond the hazardous nitrite and hydroxylamine intermediates to usable ammonia.¹¹¹

Vitamin A supplements have had a protective effect against nitrate toxicity in lambs but not in adult sheep.¹¹¹ Ascorbic acid has also been shown to have a protective action against nitrate toxicity. In addition, ruminants are better able to tolerate nitrates when urea is added to the ration.¹¹¹

The effects of nitrosamines and their formation within the gastrointestinal tract have been studied chiefly in laboratory animals (see Section 3.2.3). No information is available regarding the effects of nitrosamines on or their formation within the intestinal tracts of domestic animals such as cattle, hogs, horses, or sheep.

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4. CHEMISTRY AND BIOCHEMISTRY OF N-NITROSO COMPOUNDS

4.1 INTRODUCTION

Organic nitroso compounds contain a nitroso group ($-N=O$) that is attached to a nitrogen (N-nitroso) or a carbon atom (C-nitroso). The N-nitroso compounds have been known for years to be highly toxic chemicals, and many have been studied extensively since 1956 when Magee and Barnes¹ reported that N-nitrosodimethylamine (dimethylnitrosamine) produces primary hepatic tumors in rats. In contrast, the C-nitroso compounds have been of little interest.

The carcinogenic N-nitroso compounds can be conveniently divided into two groups, the N-nitrosamines and the N-nitrosamides. The N-nitrosamines consist of the dialkyl, alkylaryl, diaryl, and various cyclic nitrosamines. N-Nitrosamides consist of the alkyl and aryl nitrosamides.

N-Nitrosamines are formed when amines react with nitrous acid. Amines themselves are organic compounds derived from ammonia in which one or more hydrogen atoms have been replaced by an alkyl or aryl group. Replacement by an alkyl group yields an aliphatic amine, while replacement by an aryl group gives an aromatic amine. The structures of ammonia and of primary, secondary, and tertiary amines are shown in Figure 4-1.

Primary, secondary, and tertiary amines behave differently when treated with nitrous acid. Primary aliphatic amines react to yield nitrogen and alcohols; secondary amines yield N-nitrosamines; and tertiary aliphatic amines usually form unstable salts which are destroyed on neutralization.² However, one tertiary amine, aminopyrine, yields dimethylnitrosamine and an open-ring compound when nitrosated.³ Primary aromatic amines form diazonium salts while tertiary aromatic amines undergo ring substitution with a nitroso group ($-N=O$), which attaches generally to the para position of the aromatic ring (Figure 4-2).⁴ All these reactions, despite differences in final product, involve the same initial step, which is an electrophilic attack by the nitrosonium ion from nitrous acid that results in the displacement of a hydrogen in the amine (the hydrogen on the amino nitrogen).⁵ Tertiary aromatic amines, which have no hydrogen attached to the nitrogen atom, are attacked instead at the highly reactive aromatic ring.

Nitrosation of the amides, N-alkylureas, N-arylsureas, N-acylsureas, N-alkylcarbamates, and N-alkylguanidines results in the formation of nitrosamides.

General and specific structures of some nitrosamines are shown in Figure 4-3 and those of nitrosamides in Figure 4-4. General structures of some amides that can undergo nitrosation are shown in Figure 4-5.

Some of these chemicals have been used as chemical intermediates and reagents, and some as solvents in organic synthetic chemistry. The chemistry of the nitroso compounds has been reported in detail.⁴ As only the nitroso group is common to all the nitroso compounds, the physical properties of these compounds cover a wide range. At ambient temperature, the N-nitrosamines are, depending on their structure, either liquids (e.g., dimethylnitrosamine) or solids (e.g., diphenylnitrosamine). Except for the dimethyl and diethyl derivatives, they are quite insoluble in water but soluble in many organic solvents. The boiling points of the lower *n*-alkylnitrosamines range from 150° to 220°C.

4.2 FORMATION OF N-NITROSO COMPOUNDS

N-nitroso compounds can be formed when a secondary amine and nitrite react under favorable conditions.⁶

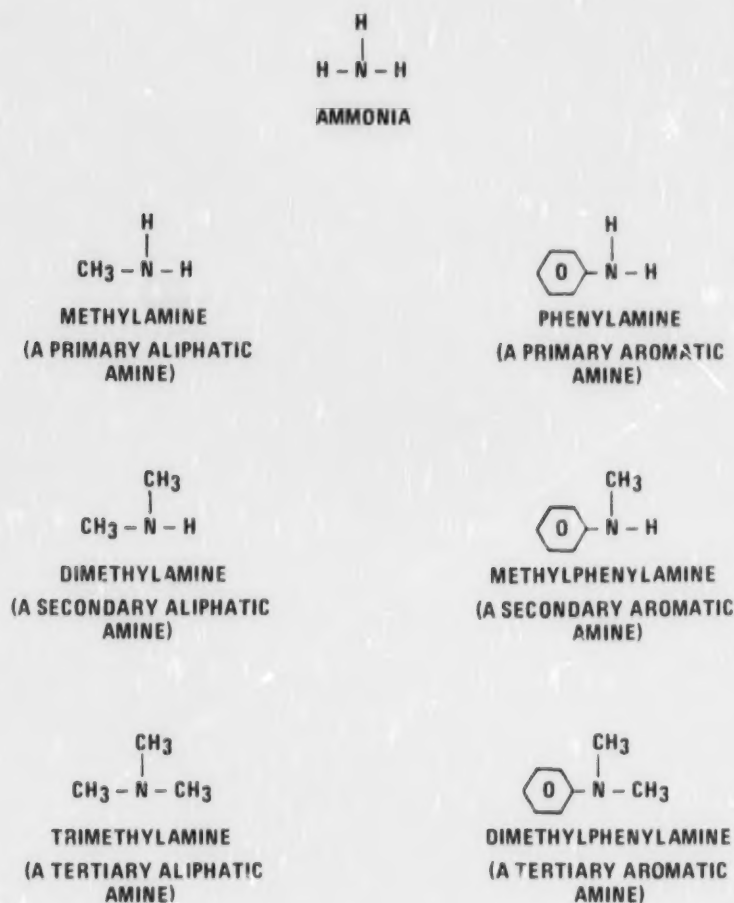
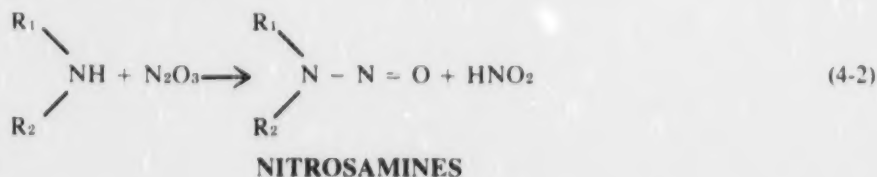


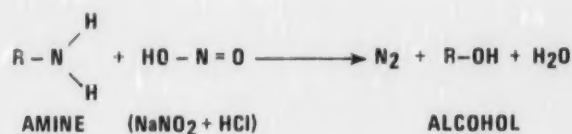
Figure 4-1. Structures of ammonia and of primary, secondary, and tertiary amines.

4.2.1 Kinetics of Nitrosation

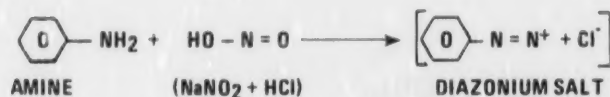
For nitrosation to occur, nitrite is usually first converted to nitrous acid ($\text{pK}_a = 3.37$), which explains why nitrosation is catalyzed by acid. The HNO_2 is then converted to an active nitrosating species, e.g., nitrous anhydride (N_2O_3), nitrosyl thiocyanate ($\text{ON} \cdot \text{NCS}$), nitrosyl halide (NOX), or nitrous acidium ion (H_2NO_2^+).

Most secondary amines are nitrosated according to the following equations:⁶

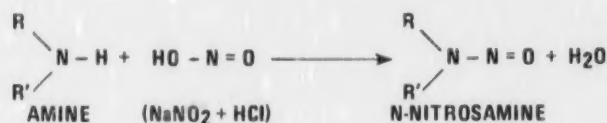




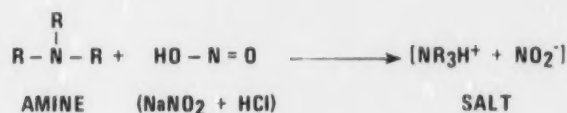
a. Primary aliphatic amine and nitrous acid.



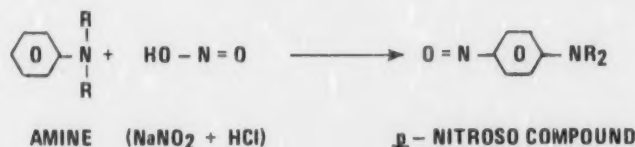
b. Primary aromatic amine and nitrous acid.



c. Secondary aliphatic amine and nitrous acid, where R and R' may be alkyl or aryl groups or may represent carbon atoms in a ring structure.



d. Tertiary aliphatic amine and nitrous acid.



e. Tertiary aromatic amine and nitrous acid.

Figure 4-2. Reactions of various amines with nitrous acid.

The nitrosating agent is nitrous anhydride (N₂O₃) produced from two molecules of nitrous acid. The rate of nitrosation is proportional to the concentrations of the amine and the N₂O₃ and hence to [HNO₂]².^{*} The rate equation can therefore be expressed as:

$$\text{Rate} = K_1 \times [\text{R}_2\text{NH}] \times [\text{HNO}_2]^2 \quad (4-3)$$

or

$$\text{Rate} = K_2 [\text{total amine}] \times [\text{total nitrite}]^2 \quad (4-4)$$

^{*} Brackets indicate concentrations of reactants.

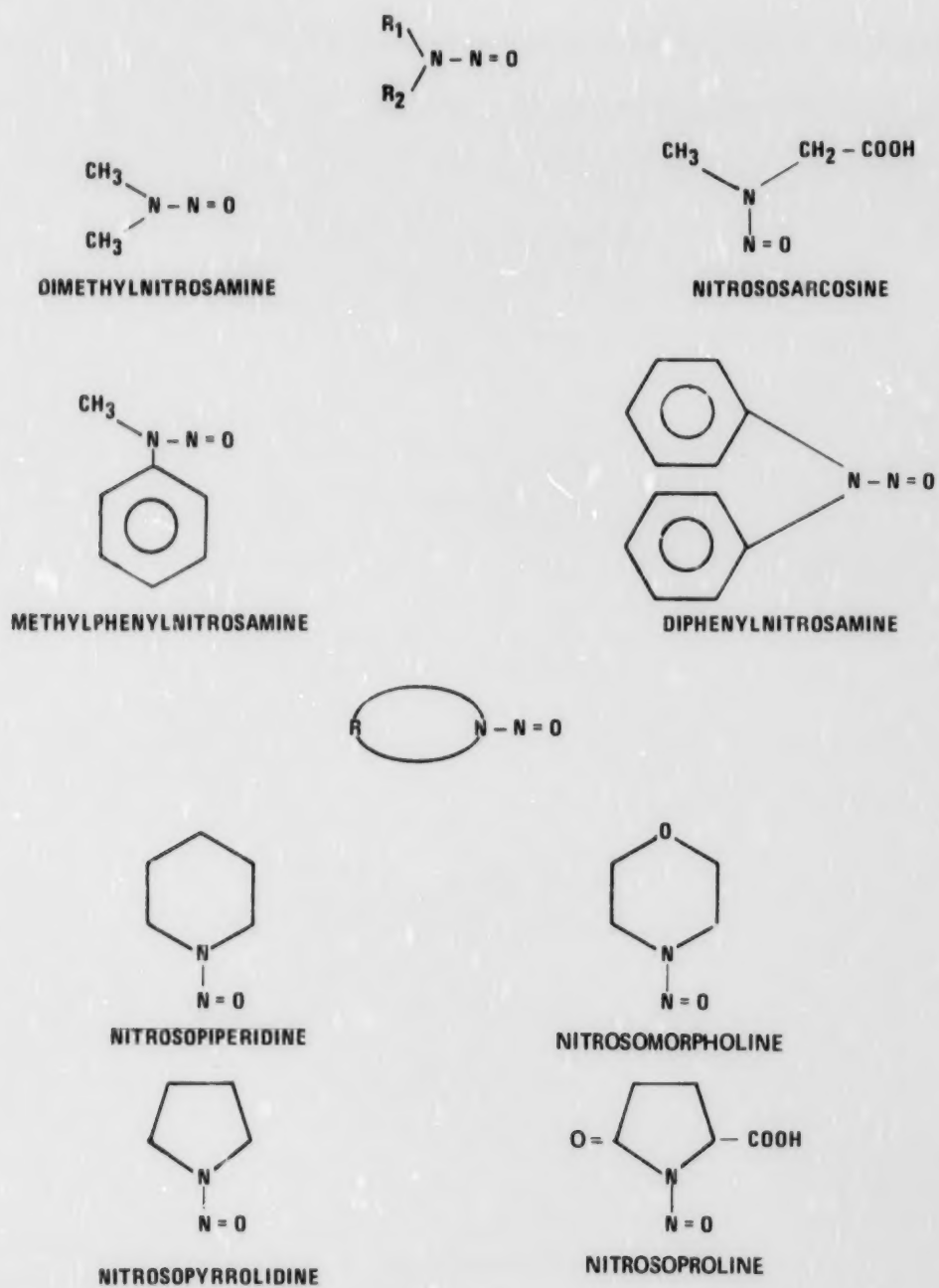


Figure 4-3. Structures of various N-nitrosamines (R₁ is an alkyl or aryl radical and R₂ may be one of many other functional groups).

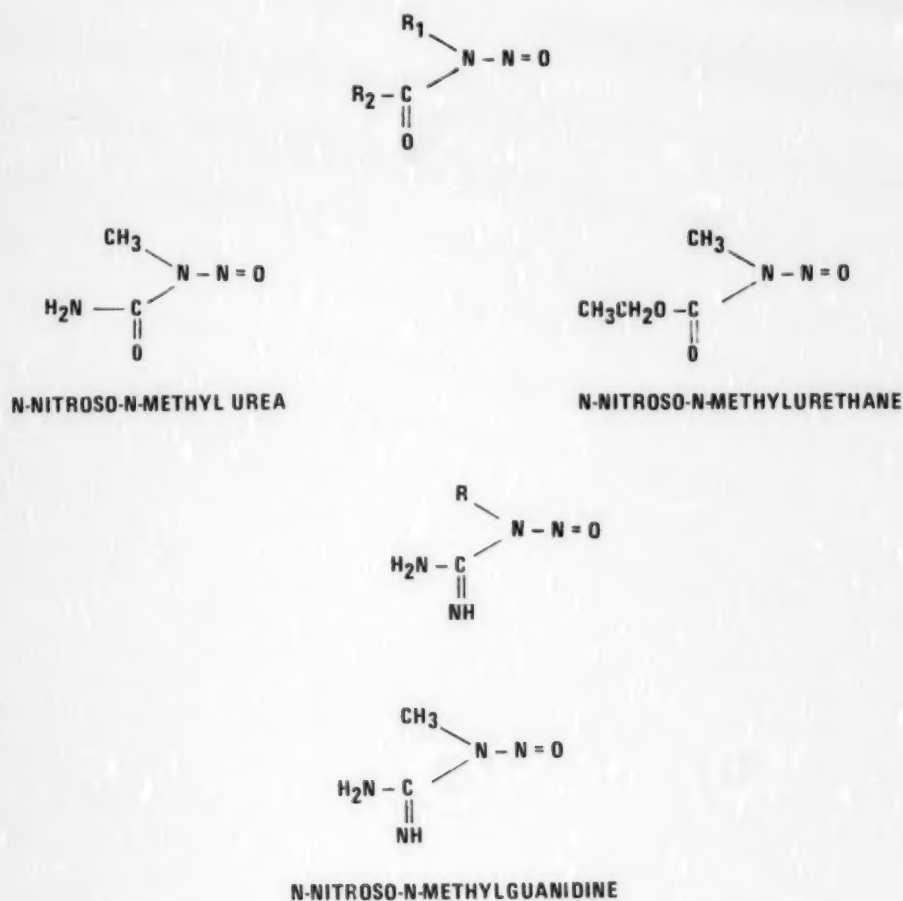


Figure 4-4. Structures of various N-nitrosamides (R_1 is an alkyl or aryl radical and R_2 may be one of many other functional groups).

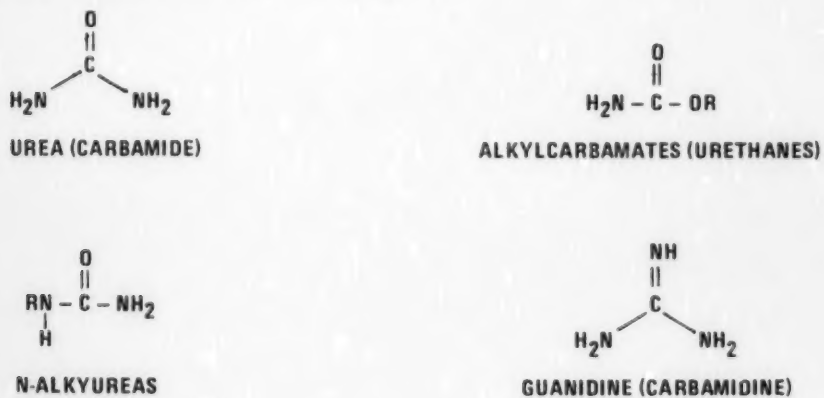


Figure 4-5. General structures of some amides that can undergo nitrosation.

where $[R_2NH]$ and $[HNO_2]$ are the concentrations of the nonionized amine and the free nitrous acid, respectively, and hence have values dependent on the total amine and total nitrite concentrations as well as on pH. In equation (4-3), although the nonionized amine and free nitrous acid both vary with pH, the K_1 is independent of pH. Equation (4-4) is easier to apply, since the total concentrations of amine and nitrite are used, irrespective of the actual species present; but here the stoichiometric rate constant K_2 varies with pH.^{7,8} The reaction rate and K_2 show maximum values at pH 3.4, the pK_a for nitrous acid.⁹ The rate constants K_1 and K_2 for 14 secondary amines and 1 tertiary amine are listed in Table 4-1. The ease of nitrosation, as given by K_2 , increases as the basicity of the amine decreases, i.e., as pK_a decreases.¹⁰ Thus, the weaker bases will be nitrosated more rapidly because of the higher relative concentration of free amine at all values of pH below their pK_a .

Table 4-1. RATE CONSTANTS K_1 AND K_2 (EQUATIONS 4-3 AND 4-4) FOR THE NITROSATION OF AMINES AT OPTIMUM pH AND 25°C

Amine	pK_a^{23}	K_2 , $M^{-2} \text{ sec}^{-1}$	$K_1 \times 10^{-5}$, $M^{-2} \text{ sec}^{-1}$	Ref.
Secondary amines				
Piperidine ^a	11.2	0.00045	1.4	10
Dimethylamine ^a	10.72	0.0017	1.5	7
Pyrrolidine ^a	11.27	0.0053	21.0	6
N-Methylethanolamine	9.5	0.010	0.62	6
N-Methylbenzylamine	9.54	0.013	0.92	6
Proline ^b	-	0.037	1.4	11
Sarcosine ^b	-	0.23	2.6	11
Prolylglycine	8.97	0.25	5.0	11
Hydroxyproline ^{a,b}	-	0.31	2.1	11
Prolylleucylglycineamide	8.97	0.38	6.2	11
Morpholine	8.7	0.42	2.3	14,10
Mononitrosopiperazine	6.8	6.7	0.83	10
Piperazine	{ 5.57 9.8	83	0.62	10
N-Methylaniline ^c	4.85	250	18.0	13
Tertiary amine				
Aminopyrine ^c	5.04	80	1.0	12

^a Naturally occurring.

^b No pK_a given because of complex ionization.

^c Examined at 0°C.

The only tertiary amine whose kinetics have been examined is the analgesic, aminopyrine,¹² which is rapidly nitrosated to give dimethylnitrosamine^{3,15} and the ring-opened compound, 1-diketobutyl-1-phenyl-2-methyl-2-nitrosohydrazide,¹² as shown in Figure 4-6. The optimum pH for dimethylnitrosamine formation was 2.0, the kinetics can be expressed as:

$$\text{Rate} = K_3 [\text{HNO}_2]^2 \quad (4-5)$$

The ease of nitrosation of aminopyrine is attributed to its enamine structure and low pK_a .

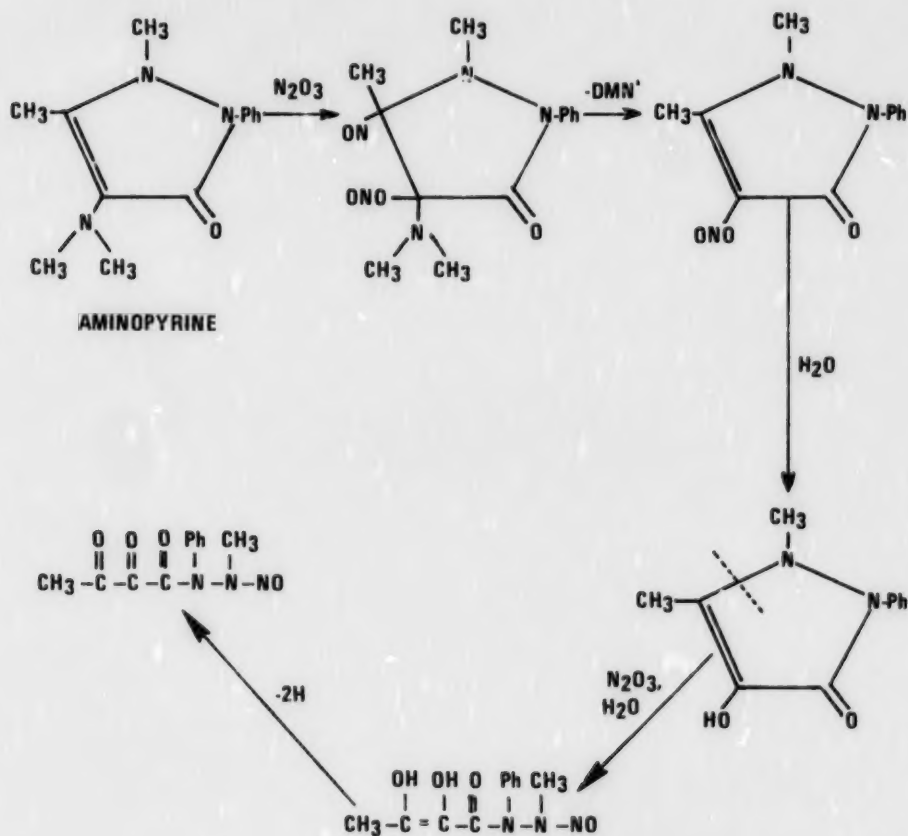
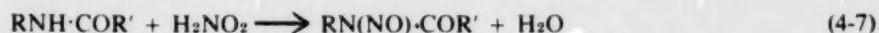


Figure 4-6. Nitrosation of aminopyrine to give dimethylnitrosamine and 1-diketobutyl-1-phenyl-2-methyl-2-nitrosohydrazide.¹²

Nitrosation of ureas, guanidines, and carbamates may be important because (1) at least 14 ureas and 8 guanidines are found in nature, where the ureas probably arise by the bacterial deimidation of guanidines or carbamylation of amines;¹⁶ and (2) many ureas and carbamates are used as drugs and, often because of their anticholinesterase activity, as insecticides. N-alkyl ureas and carbamates are rapidly nitrosated at pH 1 to 2.^{17,18} For nitrosation of these and other amides (equations 4-6 and 4-7), the reaction rate increases about 10 times for each 1-unit drop in pH from 3 to 1, does not show a pH maximum, and follows the rate equation expressed in (4-8) and (4-9).¹⁶



$$\text{Rate} = K_6 [\text{RNH}\cdot\text{COR}'] [\text{HNO}_2] [\text{H}^+] \quad (4-8)$$

$$\text{Rate} = K_7 [\text{amide}] [\text{nitrite}] [\text{H}^+] \quad (4-9)$$

The main nitrosating agent is probably nitrous acidium ion (H_2NO_2^+ , protonated nitrous acid). The nitrosation rate is proportional to the [amide] and $[\text{HNO}_2^+]$ (equation 4-7), which in turn is proportional to $[\text{H}^+]$ and $[\text{HNO}_2]$ (equation 4-6). Thus the rate equations (4-8) and (4-9) apply. In equation (4-8), K_6 depends on nitrite ionization and hence on pH, but does not depend on ionization of the amides since these are usually nonionized above pH 2; e.g., methylurea has a pK_a of 0.7. The reaction of benzamide and acetamide with HNO_2 probably also proceeds via H_2NO_2^+ , which may be favored for amide nitrosation because of electrostatic interaction with the amidic oxygen.¹⁹

Nitrosation of the 21 amides in Table 4-2, including 14 ureas, 2 carbamates, and 2 guanidines, followed equation (4-8). The K_6 values varied 300,000 times between different amides. They were lowest for simple alkyl- and arylamides and for guanidines; next lowest for the acylureas, dihydrouracil, and hydantoin; and highest for 2-imidazolidone (ethylene urea). Unlike for the amines, there is no simple rule relating ease of nitrosation to other properties of the amide.

In summary, the kinetic results show that N-alkylureas, N-arylureas, N-alkylcarbamates, secondary aromatic amines, secondary amine derivatives of piperazine and morpholine, and tertiary enamines are more readily nitrosated than simple aliphatic secondary and tertiary amines, N-acyl-

Table 4-2. RATE CONSTANT K_6 (EQUATION 4-8) FOR THE NITROSATION OF AMIDES AT 25°C AND pH 2

Amide	K_6 , $\text{M}^{-2} \text{sec}^{-1}$	References
N-Methylbenzamide	0.0014	6
N-Methylacetamide	0.0025 ^a	6
Dihydrothymine ^b	0.0035	6
Methylguanidine ^b	0.004	17
1-Methyl-3-nitroguanidine	0.008	6
Dihydrouracil ^b	0.010	6
Hydantoin	0.042	10
Ethyl N-ethylcarbamate	0.10	—
Hydantoic acid ^b	0.18	6
Ethyl N-methylcarbamate	0.37	17
2-Ureidoethanol	0.38	6
DL-Citrulline ^b	0.72	—
Phenylurea	2.2	6,20
Ethylurea	3.0	17
Trimethylurea	7.0	6
Ethylcyanamide	8.0	6
Methylurea	10.5	17
Ethoxyphenylurea	13	6
2-(1H)-Tetrahydropyrimidinone (propylene urea)	18	6
1,3-Dimethylurea	200	6
2-Imidazolidone (ethylene urea)	400	6

^a Measured at pH 1.

^b Naturally occurring.

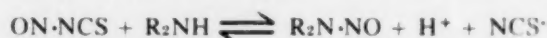
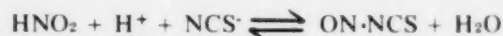
ureas, and N-alkylguanidines. Thus, the former classes of compounds are of more interest than the latter classes as potential precursors of human carcinogens. However, potential carcinogenicity depends on the relative amount of compound to which man is exposed.

4.2.2 Factors That Influence Nitrosation

The acidity prevailing in the mammalian stomach favors the formation of nitrosamines from amines and nitrites. Sander and his colleagues^{21,22} showed the formation of the corresponding N-nitroso compound by incubating several secondary amines (e.g., diphenylamine, N-methylaniline, and N-methylbenzylamine) with nitrite in the presence of gastric juice under various conditions. Sen et al.²³ demonstrated the *in vitro* formation of diethylnitrosamine and sodium nitrite in the gastric juice from rats, rabbits, cats, dogs, and man. Eisenbrand et al.²⁴ showed the formation of dimethylnitrosamine in the stomachs of rats given aqueous solutions by stomach tube of sodium nitrite and one or the other of the fungicides, ziram ($[(CH_3)_2N-CS-S]_2Zn$) and ferbam ($[(CH_3)_2N-CS-S]_3Fe$). Sander and Seif²⁵ showed the formation of nitrosodiphenylamine ($1\ \mu g$ to $5\ \mu g$) in the stomachs of people given nitrite and diphenylamine.

In the simplest case of nitrosamine formation, the rate of reaction is proportional to the square of the nitrite concentration and directly proportional to the concentration of the unprotonated amine. This simple situation is greatly complicated by the additional chemical and physical components and factors in the actual reaction environment, some of which can be listed as follows:

- Not all nitrosatable substances follow the same kinetic pattern, even in simple reaction systems. For example, amides and ureas follow a completely different rate law¹⁷ from other nitrosatable compounds, and the optimum pH for nitrosation of amino acids and peptides is different from that for amines.¹¹
- Many reactions of interest are multistep processes; e.g., the formation of N-nitrososarcosine from creatine²⁶ or N-nitrosodimethylamine from trimethylamine.^{27,28} In each of these cases, nitrite is a reactant in more than one step, which leads to a complicated kinetic pattern. In other cases, e.g., the formation of N-nitrosopyrrolidine from spermidine, even the reaction mechanism is not yet understood.²⁹
- A number of chemical substances can accelerate the rate of nitrosamine formation. Thiocyanate increases the rate of nitrosation of morpholine, N-methylaniline, aminopyrine, and sarcosine^{11,30} because the nitrosating species ON·NCS is produced. The kinetics follow equations (4-10) and (4-11).⁴ The optimum pH for nitrosation at high $[NCS^-]$ is 2.0, when nitrite becomes fully protonated. Below pH 2, the rate falls because NCS^- becomes protonated. Bromide and chloride have effects similar to those of NCS^- , due to the formation of NOBr and NOCl; but the rate does not fall below pH 2. The order of activity is $NCS^- \gg Br^- > Cl^-$.⁸ For sarcosine nitrosation under specific conditions, addition of NCS^- increased the reaction rate by a factor of 400 at pH 0.5, 100 at pH 1.5, and 10 at pH 2.5.¹¹ The reaction of nitrous acid with thiocyanate proceeds as shown:



$$\text{Rate} = K_4 [R_2NH] [HNO_2] [H^+] [NCS^-] \quad (4-10)$$

or, since $[R_2NH] [H^+]$ is independent of pH,

$$\text{Rate} = K_5 [\text{amine}] [HNO_2] [NCS^-] \quad (4-11)$$

Thus thiocyanate- and halide-catalyzed nitrosation of amines (4-10) and (4-11) will compete favorably with the N_2O_3 mechanism (4-3 and 4-4) under three conditions: (1) at pH < 2.5, where reaction by (4-4) but not by (4-11) begins to slow down; (2) at high $[NCS^-]$ or $[\text{halide}]$; and (3) at low $[\text{nitrite}]$, where reaction by (4-4) drops sharply because it is proportional to

[nitrite]², whereas reaction by (4-11) is proportional only to [nitrite].¹⁴ Since attack by N₂O₃ (ON·ONO), ON·NCS, and NOX are similar reactions, the order of amine reactivity according to (4-4) (Table 4-1) probably also applies to nitrosations catalyzed by NCS⁻ and halides.

The thiocyanate ion could affect intragastric nitrosation since it occurs in saliva, especially that of smokers (which has about 6 mM NCS⁻), and in gastric juice (0.2 to 0.7 mM NCS⁻).³¹ It should therefore not be surprising if the kinetic pattern of nitrosation in stomach contents is complex indeed.^{10,32}

In another mechanism of acceleration recently discovered, formaldehyde can alter the mechanism of nitrosation to enable nitrosation in neutral or basic media,³³ conditions that should ordinarily lead to an infinitesimally small amount of nitrosamine. The mechanism of the formaldehyde-catalyzed nitrosation of various secondary amines at pH 6 to 11 can be written as shown in Figure 4-7.

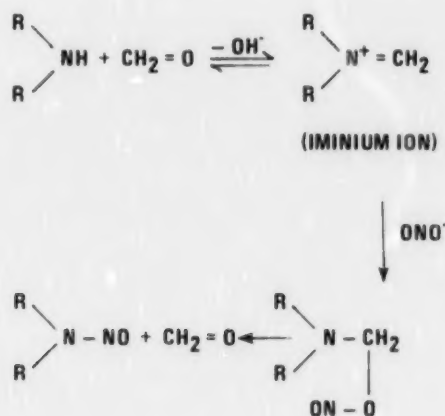


Figure 4-7. Formaldehyde-catalyzed nitrosation of secondary amines at pH 6 to 11.

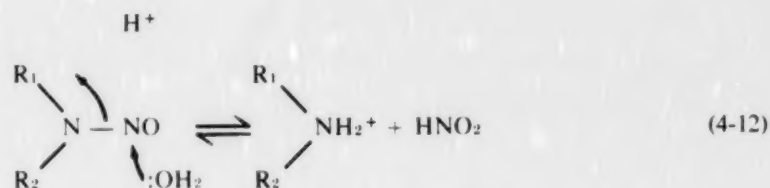
Catalytic effects on nitrosation by sulfate and phosphate have also been shown.¹⁷

- Other chemical substances retard the rate of nitrosation, e.g., tannins,³⁴ sulfhydryl compounds,³⁵⁻³⁹ and ascorbic acid.⁴⁰ Nitrite can react with various secondary amines in the acidic environment of the stomach to form nitrosamines.^{23,25,35} Ascorbic acid reacts rapidly with nitrite under the same acidic conditions to form nitrosoascorbate as an intermediate.⁴⁰ Ivankovic et al.⁴¹ have shown that ascorbic acid inhibits the teratogenic and carcinogenic effects of ethylnitroso urea⁴² formed in the gastrointestinal tract of rats. These results confirm other studies³⁵⁻³⁸ which have shown that ascorbic acid given concurrently with precursor amines and nitrite prevents the acute toxic effects consequent to the formation of N-nitroso compounds in animals. Recent studies by Sen and Donaldson³⁵ have indicated that glutathione is more efficient than ascorbic acid in inhibiting the formation of nitroso-piperazines from piperazine adipate and nitrite.

4.3 DEGRADATION OF N-NITROSO COMPOUNDS

4.3.1 Decomposition

Nitrosamines are chemically stable under physiological conditions and in strong alkaline medium; but under acid conditions, they undergo photodecomposition to yield nitrous acid and the parent amine.⁴³ The hydrolytic reaction for dialkyl nitrosamine can be expressed as follows:



It is assumed that water and proton act in a concerted manner to cleave the N-N bond in the N-nitrosamine. Nitrous acid in an aqueous solution is fairly stable and is a good nitrosating agent in the acidic pH range.⁴⁴ Thus, recombination of the parent amine with nitrous acid to regenerate the dialkyl-nitrosamine also occurs at a considerable rate. Under basic conditions, photolysis yields substantial amounts of nitrite ion (NO_2^-). When exposed to ultraviolet light, N-nitrosamines decompose, resulting in the quantitative formation of nitrite. This decomposition reaction has been used in one of the methods for estimating N-nitrosamines.⁴⁴ While the N-nitrosamines are stable at alkaline pH, the N-nitrosamides are unstable under such conditions and may decompose even at neutrality to form powerful alkylating agents, the diazoalkanes. The half-life of the N-nitrosamide, N-nitrosomethyl urea, for example, is 125 hours at pH 4.0, 24 hours at pH 6.5, 1.2 hours at pH 7.0, and only 0.1 hour at pH 8.0.⁴⁵ Preussmann and Schaper-Druckrey⁴⁶ have shown that in acid nitrosamides decompose to give quantitative yields of nitrite. These differences in chemical stability may underlie the differences in biological behavior of the nitrosamines and the nitrosamides since there is evidence that a decomposition product rather than the parent molecule may be responsible for the biological action of these compounds. It has been suggested that the nitrosamines require enzyme-catalyzed decomposition before they become biologically active; whereas the nitrosamides can decompose in the tissues without enzymatic activation.⁴⁷

4.3.2 Metabolism

N-nitroso compounds are relatively rapidly metabolized in whole animals. In the rat or mouse, for example, the administration of ^{14}C -labeled dimethylnitrosamine leads to the majority of the isotope appearing as $^{14}\text{CO}_2$ after 6 to 10 hours.⁴⁸ In similar experiments with ^{15}N -labeled dimethylamine, the isotope appeared in urea and proteins, indicating that the nitroso group as well as the amino group is transformed metabolically into ammonia, which is in equilibrium with the endogenous ammonia "pool" of the animal.⁴⁹ Thus the carcinogenic N-nitroso compounds can be metabolized to a large extent by pathways which are of importance to the normal animal.

Montesano and Magee⁵⁰ have shown that dimethylnitrosamine and diethylnitrosamine are metabolized to varying degrees by tissue slices of several organs other than the liver. Of the tissue slices from the rat, liver was the most active in metabolizing dimethylnitrosamine; kidney showed appreciable activity; and lung and esophagus were definitely active, while the activity of small intestine was extremely low. A similar general pattern was seen with the diethyl compound, but the rate of metabolism was lower in the liver slices and higher in the lung than with the dimethyl compound.

With hamster tissues the pattern of dimethylnitrosamine metabolism was similar to that found with rat tissues. Metabolism of the diethyl compound, however, was remarkably different in that hamster lung tissue had a greater capacity than liver for metabolizing this carcinogen, while the other organs were considerably less active.

Metabolic capacity may be correlated with carcinogenicity in kind and degree. Diethylnitrosamine, readily metabolized by hamster lung tissue, is a powerful carcinogen for the hamster respiratory tract; whereas dimethylnitrosamine, less readily metabolized, shows no carcinogenicity in hamster lung at all.

Biochemical studies with human tissues have indicated that the liver metabolizes nitrosamines especially well. Evidence from such studies points to the production of an active, ultimate carcinogen by means of the metabolic degradation of the parent nitrosamine. This ultimate carcinogen has been shown to interact with significant cellular macromolecules.⁵¹ If the carcinogenicity of nitrosamines is indeed related to capacity for metabolizing the parent compound, then it is reasonable to believe that man would be sensitive to this class of carcinogens.

As the result of several years of research on the metabolism of nitrosamines, a pathway was proposed⁵²⁻⁵⁴ for the metabolism of dialkyl nitrosamines; it is shown in Figure 4-8, using dimethylnitrosamine as an example.

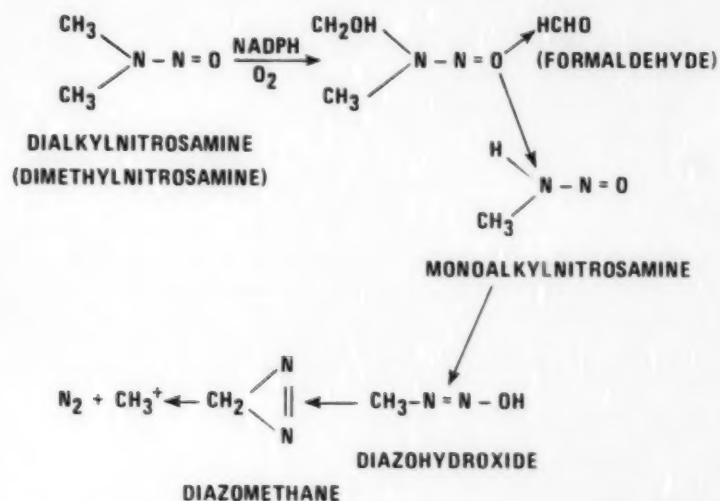


Figure 4-8. Possible pathway for the metabolism of dialkyl nitrosamines, using dimethylnitrosamine as an example.⁵²⁻⁵⁴

Dialkyl nitrosamines undergo an enzymatic hydroxylation at the α carbon in one of the aliphatic chains. Further oxidation and hydrolysis produce an aldehyde and the monoalkyl nitrosamine. It is believed that the monoalkyl nitrosamine rearranges to the corresponding diazohydroxide, which becomes the diazoalkane. This diazoalkane then decomposes to nitrogen and the alkyl carbonium ion, the latter alkylating nearby nucleic acids and other macromolecules. No evidence of the production of a diazoalkane in this way has been presented, although this possibility has been widely postulated.⁵²⁻⁵⁵ Recently Lijinsky and coworkers⁵⁶ have shown that in the case of alkylation of nucleic acids by dimethylnitrosamine alkylation is a transmethylation with no exchange of hydrogen atoms involved in deriving the methonium ion. This, then, argues against the formation of diazomethane as an intermediate in dimethylnitrosamine metabolism. Ethylation of nucleic acids has been demonstrated after administration of diethylnitrosamine,⁵⁷ which supports α -oxidation of one ethyl group and transethylation of the second.

Recently Kruger⁵⁸ has provided evidence that β -oxidation of dialkyl nitrosamines may also occur, in a manner that resembles β -oxidation of fatty acids. The first step would require enzymatic dehydrogenation between the α and β carbons of one of the alkyl chains. Adding water to the double bond produces the β -hydroxylated nitrosamine, because the nitroso group would have an inductive effect that would be analogous to the effect the activated carbonyl group has in the β -oxidation of fatty acids.

Oxidation of the hydroxylated nitrosamine would continue, yielding acetyl-CoA and methylalkyl nitrosamine; the nitrosamine might then undergo α -oxidation, producing either the methonium ion or the carbonium ion corresponding to the second alkyl group. Studies on the metabolism of [¹⁴C]di-*n*-propylnitrosamine labeled in the 1 or 2 position and [1-¹⁴C]di-*n*-butylnitrosamine support this pathway.

4.4 ATMOSPHERIC CHEMISTRY OF NITROSAMINES

For the assessment of possible N-nitrosamine pollution in ambient air, the chemistry of major interest is that pertaining to the stability in air of these compounds and to their presence in ambient and source atmospheres. Although the presence of N-nitrosamines in ambient air has been reported by Bretschneider and Matz,⁵⁹ Fine et al.,^{60,61} Pellizzari,⁶² and by researchers at Dupont,⁶³ the amounts of N-nitrosamines detected were small. Information on the stability and formation of N-nitrosamines in the gas phase, particularly in ambient atmospheres, is sparse.

The various members of the N-nitrosamine family range from high-vapor-pressure liquids to rather non-volatile solids (at room temperature), but they are capable of emitting vapor at concentrations sufficiently high to exert adverse health effects.⁶⁴⁻⁶⁶

There is no evidence of thermal instability of nitrosamines in the gaseous phase. In the condensed phase, nitrosamines are stable in an alkaline or mildly acidic environment, decomposing hydrolytically only in prolonged contact with strong acid. Thus, although acidic aerosols are known to exist in ambient air under normal atmospheric conditions, they are not believed to cause significant thermal degradation of nitrosamines.

Under ultraviolet and visible light, N-nitrosamines undergo photochemical decomposition with subsequent split-off of the nitrosyl group. This photolytic decomposition may be a factor limiting the lifetime of nitrosamines in the atmosphere.⁵⁹ The rate of such photochemical denitrosation is known to vary from compound to compound, but quantitative data are limited.^{64,66}

The chemical formation of nitrosamines has been the subject of numerous studies that have recently been reviewed by Mirvish.⁶ Although most of the reported studies have been concerned with condensed-phase reaction systems, the formation of nitrosamine in the gas phase has been demonstrated.^{59,67} Neurath et al.⁶⁷ showed that the formation of nitrosamines from secondary amines requires an equimolar mixture of nitrogen dioxide. This reaction, which occurs in the gaseous phase, can be represented as follows:



Bretschneider and Matz⁵⁹ showed that diethylamine and nitrogen dioxide (NO_2) at concentrations of 50 to 100 parts per million (ppm) reacted within seconds to form measurable levels of nitrosamine. In some cases, as in tobacco smoke, nitrosamines can form so readily that their formation can take place during the collection step.⁶⁸

It now appears that a much more intense look will have to be taken at the concentration of nitrosamine precursors in polluted atmospheres and in the background chemical environments of high-risk groups. Thus, from a genotoxic viewpoint, knowledge of the environmental concentrations of nitric oxide, nitrogen dioxide, nitrous acid, nitrites, nitrates, and primary, secondary, tertiary, and quarternary amines will be required. In addition, knowledge of the nitrosamine cocarcinogens and the catalysts favorable to nitrosamine formation will also be necessary.

Some work with gas-phase systems is currently being conducted by EPA.⁶⁹ In this study, gaseous dimethylamine, $(\text{CH}_3)_2\text{NH}$, has been shown to react with gaseous nitrous acid, HONO, in air to yield N-nitrosodimethylamine, $(\text{CH}_3)_2\text{N-NO}$. This on-going research has shown that, in a humid atmosphere containing dimethylamine, NO, NO_2 , and HONO at concentrations of 0.5 to 2 ppm, the amine reacted at a rate of about 4 percent per minute yielding N-nitrosodimethylamine as the major reaction product. In the absence of HONO and humidity, the rate was lower by a factor of four, approximately. The N-nitrosodimethylamine decomposed in sunlight with a half-life of about 30 minutes. Long-path infrared absorption spectroscopy was used to monitor these reactions at parts-per-million levels.

Assuming a bimolecular second-order reaction, an upper-limit rate constant of $0.1 \text{ ppm}^{-1} \text{ min}^{-1}$ was calculated for the reaction of dimethylamine with HONO. Such a rate indicates that dimethylamine released into a polluted urban atmosphere could be nitrosated during the night. The extent of the nighttime nitrosation would depend upon the NO, NO_2 , and H_2O concentrations. For example,

in an atmosphere containing 0.18 ppm NO₂ and 20,000 ppm H₂O, the equilibrium concentration of HONO would be 0.01 ppm, and dimethylamine would react at a rate of about 6 percent per hour. The nitrosation rate and accumulated nitrosamine concentration would diminish rapidly after sunrise as a result of the decomposition of nitrosamine and HONO by sunlight.

The homogeneous gas-phase formation of nitrosamine has been questioned by Glasson,⁷⁰ however. Although his results do confirm that the dimethylamine disappears at the rate observed by the EPA group, the formation rate of nitrosamine is less than 1 percent of the loss rate of dimethylamine. This suggests that the formation of nitrosamine is very slow and that the reaction is not homogeneous. The observations made by Glasson are compatible with a theoretical calculation made by EPA of the rate constant for the formation of nitrosamine from the reaction of dimethylamine with HONO.

Recent work by Fine⁶¹ has indicated that under certain conditions dimethylamine can be converted to dimethylnitrosamine. Fine found high conversions of dimethylamine to dimethylnitrosamine when sampling with a cold-trap collection system under conditions of high ozone levels in the presence of NO_x and dimethylamine. At the time of this report, it has not been established whether Fine's observation is due to an artifact formed within the cold-trap collection system or is the result of a gas-phase reaction. Based, however, on theoretical calculations made by EPA for the reaction of dimethylamine in the presence of O₃ and NO_x, it appears that this reaction cannot occur homogeneously.

The bulk of available evidence indicates that the ease of nitrosation of the amine can be influenced by amine basicity,²² substrate concentration,⁷ thiocyanate ion,⁷¹ chloride ion,^{6,72} acetate ion,⁸ formaldehyde,³³ readily oxidized phenolic compounds,⁷³ and pH.⁷ Assuming optimum pH conditions do occur on the surface of ambient aerosol particles, the rate of nitrosation of dimethylamine in ambient air can be calculated from the existing kinetic data to be:

$$\text{Rate}_{\text{dimethylamine}} = [\text{nitrite}]^2 \text{ percent/hour} \quad (4-14)$$

where [nitrite] is the concentration (molarity) of total nitrite on the aerosol particle surface. For more reactive amines, the rate can be higher by more than four orders of magnitude. Because nitrate concentrations in ambient air are uncertain, accurate calculations of the nitrosamine concentrations expected to form in air cannot be made. However, the rate data presented here and the fact that ambient air aerosol contains a significant concentration of nitrite suggest that formation of nitrosamines in atmospheres containing acidic aerosol and elevated secondary amine concentrations, e.g., around amine emission sources, may be important.

To summarize the information available on atmospheric chemistry of nitrosamines, it appears that atmospheric reactions constitute a probable source of ambient nitrosamines, but that they also include nitrosamine destruction steps. The formation process may include heterogeneous as well as homogeneous gas-phase reaction steps; the relative importance of the respective steps is unknown. Precursors in the heterogeneous process are the amine and, in all probability, nitrite, aerosol, and catalytic agents (e.g., halides). Also, precursors in the homogeneous gas-phase process are the amine and the nitrogen oxides or one or more nitrogen oxide derivatives (e.g., N₂O₃, NHO₂). The main atmospheric destruction process is probably sunlight-induced photolysis. Overall, the information available has only qualitative validity. The magnitudes of the formation and destruction processes and the dependence of these processes on the various factors are either totally unknown or are known with very little confidence.

In the face of these uncertainties, the existing information on the atmospheric chemistry of nitrosamines can have only vague implications regarding the control of atmospheric nitrosamines. Thus, to decide whether the nitrosamine problem is an ambient-atmosphere problem or an industrial-atmosphere problem requires that the nitrosamine-to-precursor dependencies be better known. If the problem is one of nitrosamine pollution in the ambient atmosphere, then the question of control becomes extremely complex, requiring consideration of a multitude of factors, some of which may be of non-atmospheric nature. In addition, deliberations regarding relative control requirements for amine and NO_x emissions should include consideration of occurrence and relative levels of these two precursors in the human body. For example, if relatively high levels of amines occur in the

human body, then NO_x emission control may be relatively more effective in suppressing nitrosamine formation both in the ambient air and in the human body.

The atmospheric chemistry studies to date are preliminary, were conducted with high concentrations of the reactants, and have not assessed whether other pollutants such as fine particulates and sulfur oxides have any significant influence on the formation and degradation of nitrosamines. Much additional research is required to evaluate the significance of secondary formation mechanisms and to determine which precursors, if any, should be controlled to reduce nitrosamine levels most effectively.

4.5 WATER AND SOIL CHEMISTRY OF NITROSAMINES

Recently environmental chemists have directed their attention to the possible formation of nitrosamines in water supplies and soils. One proposal is that these compounds in water and soil environments may result from the reaction of nitrite with amines, such as those derived from pesticides. Tate and Alexander⁷⁴ have reported the formation of dimethylamine and diethylamine in soil treated with dimethyldithiocarbamate (dibam) and diethyldithiocarbamate. The current study of Wolfe⁸⁹ may provide some insight into the possible formation of nitrosamines in drinking water.

4.6 FOOD CHEMISTRY OF NITROSAMINES

Amine precursors such as proteins, phospholipids, and amino acids are present in foodstuffs and may be available for reaction with nitrites to form nitrosamines. Nitrites are used to cure meats and fish. They are also commonly found in vegetables such as spinach, lettuce, celery, and beets, as well as in many water supplies. Secondary amines have been found in fish, vegetables, and fruit juices, although the concentrations may vary widely.^{75,76} Fiddler et al.²⁸ found that quarternary ammonium compounds such as choline, acetylcholine, carnitine, betaine, and some tertiary amines formed trace amounts of nitrosamines.

Archer et al.²⁶ found that carcinogenic nitrososarcosine can be formed from nitrite and creatine, which are present in vegetables, milk, and meat. Keybets et al.⁷⁷ found significant concentrations of nitrite in spinach after storage, but found no evidence of nitrosamines under normal conditions. When diethylamine was added and the pH lowered to about 3, a trace of diethylnitrosamine was found. Nitrosation generally occurred below pH 4.5; however, with high nitrite concentrations and a long reaction time, nitrosamines were formed even at pH 7.6. Using a tritium-labeled amine in buffered aqueous solutions, Mirvish⁷ found that the formation of dimethylnitrosamine was directly proportional to the dimethylamine concentration and to the square of the nitrite concentration. The maximum yield of nitrosamines occurred at pH 3.4. Based upon hypothetical calculations, Mirvish concluded that the concentration of dimethylnitrosamine formed would be too low to be carcinogenic. Nitrosation depends upon the basicity of the amine, and may increase a thousandfold as the basicity decreases from dimethylamine to aromatic amines. Mirvish⁷ also found that ascorbate effectively inhibited the nitrosation of amines by nitrite. Ender and Ceh⁷⁸ found that the amount of nitrosamines formed increased with temperature and time of reaction, but that significant nitrosation could occur even at -18°C .

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5. SAMPLING AND ANALYTICAL TECHNIQUES FOR NITROSAMINES

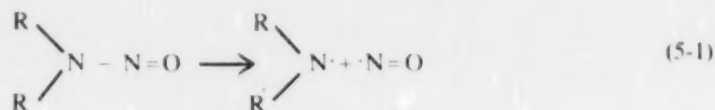
5.1 AIR

Until recently, the detection of low concentrations of nitrosamines, such as those expected in the environment, has been difficult because of the general properties of these compounds.¹ For example, they do not fluoresce, they do not have well-defined absorption spectra, and they are difficult to separate from other nitrogenous compounds.² Most of the nitrosamines found in the environment are volatile at least at steam temperature, but the majority of nitrosamines are not volatile, which makes their detection difficult.³ Furthermore, a thorough assessment of nitrosamine pollution requires not only the measurement of nitrosamines but also of their precursors — that is, amines and nitrosating agents — for which no satisfactory methods are yet available.³

Previous technology for the detection and measurement of nitrosamines was developed primarily for the assessment of food and biological samples. Recent interest in nitrosamines in ambient air instigated the development of methods specific for sampling and analysis of ambient air. The main steps in these methods are sample concentration, organic component separation, and component identification and measurement.

Fine and coworkers⁴⁻⁹ at the Thermo Electron Research Center (TERC) have developed a new, specific method for detecting N-nitroso compounds that is based upon the catalytic cleavage of the N-NO bond and the subsequent infrared detection of chemiluminescence. The technique, called thermal energy analysis (TEA) by its developers, is coupled with gas-liquid chromatography (GLC-TEA) and, for some uses, with high-pressure liquid chromatography (HPLC-TEA). This new technique is highly specific for heat-labile nitrosyl groups. The method appears to have the most acute sensitivity yet reported, so that N-nitroso compounds at the level of 1 ng/ml can be detected. In this method, air is sampled typically for 2 hours through three successive, cooled vacuum traps at a flow rate of about 2 liters/min. This freezes out the compound. The contents of the three traps are combined and stored at -78°C for later analysis. Before analysis, the combined sample is thawed and extracted three times, each time with 8 ml of redistilled dichloromethane. The combined extracts are concentrated by evaporation to a final volume of 0.5 ml, spiked with an internal standard, and chromatographed. Chromatographic separation of nitrosamines is accomplished on a 21-ft by 1/8-in. o.d. stainless steel column packed with 2 percent KOH and with 5 percent FFAP on Chromasorb WHP 80/100 mesh or with 10 percent FFAP on Chromasorb WHP 80/100 mesh. The column is operated isothermally at 185°C with a nitrogen carrier at 30 ml/min. Identification and measurement are accomplished by means of thermal decomposition of the separated nitrosamines and measurement of resultant NO by a chemiluminescence technique.⁴ The method proceeds as described below and as shown in Figure 5-1.¹⁰

1. The sample is introduced into the injection port and is subsequently separated by the chromatograph column; the TEA pyrolyzer then splits off the nitrosyl radical from N-nitroso compounds:



(R and R' may be any organic radical)

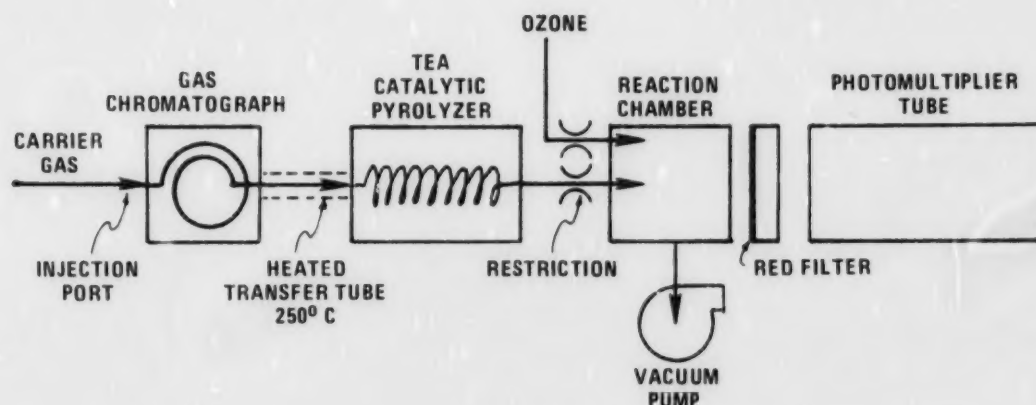
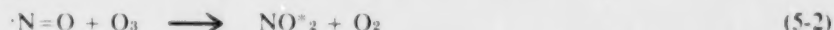


Figure 5-1. Schematic of GLC-TEA interface.¹⁰

- The effluent is expanded through a narrow constriction into an evacuated reaction chamber, where the nitrosyl radical is subsequently reacted with ozone, yielding electronically excited nitrogen dioxide:



- The excited nitrogen quickly returns to ground state, emitting light in the near infrared region of the spectrum:



This light is monitored by an infrared-sensitive photomultiplier tube; the intensity of the light is proportional to the number of nitrosyl radicals present.⁴

Calibration is linear over five orders of magnitude with GLC-TEA; therefore, quantitative analysis at the < 3 ng/ml N-nitroso compound concentration level is possible.

A practical detection sensitivity of 1 picomole of an N-nitroso compound is possible, but even greater sensitivity can be realized if the solvent is removed.

The TEA detector determines the selectivity of the method because it requires that a compound be catalytically pyrolyzed at a low temperature to give a nitrosyl radical that will react with ozone to produce infrared light. Although luminescence is also produced by the reaction of other compounds with ozone, such emissions are in the blue or visible spectrum. Despite the fact that other compounds or functional groups might react with ozone to produce an infrared luminescence, none have yet been found. In the event of such interference, the effect may be eliminated by interposing a cold trap between the catalytic pyrolyzer and the ozone reaction chamber.⁶

The solvent front, which can be observed on the chromatogram, has three distinct effects characteristics: there is an initial sharp positive peak, followed by a broad negative peak which overshoots the baseline in a positive direction before decaying back to the baseline. A cold trap, placed as previously discussed and maintained at a temperature of -15°C , eliminates the negative peak completely.⁹ This simplifies quantitation of early peaks.

Some concern over the accuracy of these methods has existed, especially with regard to the possibility that false readings of nitrosamines may result from the reaction of collected amines and nitrogen oxides during collection or analysis (artifact formation). A number of experiments conducted in the laboratory and the field have indicated that artifact formation is not a significant problem even at concentrations higher than those typically found in urban atmospheres.^{11,12} Furthermore, side-

by-side monitoring, with the TERC method and with one developed by the Research Triangle Institute (discussed below), indicates that the two completely different collection and analytical methods appear to agree rather well.^{11,12}

The HPLC-TEA method mentioned earlier is used for analysis of nonvolatile nitrosamines.⁹

A second method that is used for the analysis of nitrosamines in ambient air was developed at the Research Triangle Institute (RTI).¹¹ By the RTI method, nitrosamines are collected by passing air through a cartridge packed with solid sorbent (TENAX). Sampling times range from 1 to 5 hours. Samples are desorbed by heating into a gas chromatographic column.

For component identification and measurement, the chromatographic instrument is interfaced to a Varian Ch-7 mass spectrometer and to a Varian 620 computer for processing of the retention time and mass spectral data. The RTI method appears to be less sensitive than the GLC-TEA method developed by TERC but provides more reliable and more specific component identification data.

Because of the usefulness of both the GLC-TEA and RTI methods, and because of their differences in analytical procedure, a comparative study of the two methods was recently sponsored by the Environmental Science Research Laboratory — RTP of EPA to compare the two. The measurements were made at several locations in Baltimore where nitrosamines in the ambient air have been reported.⁷ The protocol and results of this study are shown in Table 5-1. Although the same samples were not analyzed by both techniques, the data reported from the two methods seem to agree well.

Table 5-1. PROTOCOL AND RESULTS OF COMPARATIVE STUDY OF TERC AND RTI METHODS FOR MEASUREMENT OF NITROSAMINES IN AMBIENT AIR IN BALTIMORE, MD.

Date	Sampling location	TERC method		RTI method	
		Sampling period	Nitrosamines, $\mu\text{g}/\text{m}^3$	Sampling period	Nitrosamines, $\mu\text{g}/\text{m}^3$
Oct. 15, 1975	FMC E parking lot ^a	12:17 to 1:35 p.m.	13.1	11:00 to 12:50 p.m.	3.2
		2:30 to 3:30 p.m.	14.6	3:00 to 6:50 p.m.	13.4
		4:15 to 5:45 p.m.	11.4		
		6:00 to 7:00 p.m.	8.7		
Oct. 16, 1975	Sewage plant, Patapsco Bay	11:10 to 1:10 p.m.	ND ^b	10:30 to 1:50 p.m.	ND ^b
		1:30 to 3:30 p.m.	ND	2:00 to 5:50 p.m.	ND
		3:50 to 5:50 p.m.	ND		
Oct. 17, 1975	Chesy Pier	11:00 to 1:00 p.m.	1.8	9:56 to 1:46 p.m.	0.9
		1:15 to 3:15 p.m.	1.2	2:10 to 6:00 p.m.	0.1
		3:45 to 5:45 p.m.	0.1		

^a FMC = Food and Machinery Corporation; the other sampling locations were nearby.

^b Non-detectable concentration.

5.2 WATER

5.2.1 Collection and Preservation of Samples

Although there are no known reports on collection and preservation of water samples for nitrosamines analysis, the usual precautions employed in the collection of samples for organic analysis

are recommended. Glass sample bottles should be carefully cleaned and analyzed for contaminants. The pH of the sample source should be checked prior to sample collection to see if an upward adjustment of sample pH to 8 will be required to stabilize the sample immediately after collection. The sample container should be filled completely with sample and sealed with a tight-fitting lid containing a nonporous, nonreactive liner. The sample should be protected from light, kept cold (preferably iced), and analyzed as soon as possible after collection.

5.2.2 Analytical Techniques

The search for the role N-nitroso compounds may play in the occurrence of human cancer initially led to a tremendous development and application of concentration technologies such as extraction and distillation; and of analytical technologies such as polarography, fluorimetry, photometry, chromatography, and mass spectrometry for determining these compounds and their precursors in food, beverages, and complex biological mixtures. Efforts to develop appropriate analytical procedures for determining the N-nitroso compounds in water supplies were limited, however, until 1975. Shortly thereafter Dure et al.¹³ reported the use of one-step extraction and a gas chromatographic system along with a high-sensitivity N-selective flame ionization detector to determine the levels of nitrosamines up to 100 ng/liter with a qualitative detection limit of 25 ng/liter.

Fine and his coworkers^{4-9,14,15} also reported the development and application of both a gas chromatographic system and a high-pressure liquid chromatographic system, individually combined with a catalytic pyrolyzer/chemiluminescence detection system for the determination of volatile and nonvolatile nonionic nitroso compounds, respectively, at parts-per-trillion levels in water supplies. Briefly, the procedure consists of (1) extraction of the N-nitroso compounds into dichloromethane either directly from the water or after their sorption onto carbon; (2) drying of the aqueous extracts with sodium sulfate; (3) concentration of the dried extract on a Kuderna Danish evaporator to a volume of 0.5 ml; (4) separation of the N-nitroso compounds on an appropriate gas or liquid chromatographic column; (5) elution of each N-nitroso compound individually from the column; (6) decomposition of each nitroso radical to the nitrosyl radical; (7) reaction of the latter with ozone to form excited NO₂; and (8) measurement of light emitted from excited NO₂, when it decays back to the ground state.

Recently reported developments in the application of gas chromatographic/mass spectrometric technology to the determination of the nitrosamines are encouraging. This approach may be the method of choice, for it will afford a way to positively identify nitroso compounds. Several potential compounds of interest (e.g., N-nitrosoatrazine) do not survive gas chromatography. It may be possible to derive some of these compounds, but this may reduce the reliability of the analysis.

In all of these approaches, more data are needed with which to evaluate the efficiency of the sample concentration and clean-up systems and the specificity and accuracy of the analytical systems. The possibility of both nitrosamine formation and decomposition during these procedures must also be considered.

5.3 FOOD

A variety of methods have been used to detect nitrosamines in food, such as polarography, UV absorption, thin-layer chromatography, and gas chromatography. Many of the earlier methods used for the evaluation of extractive procedures have proved to be of doubtful value because of the possibility of artifacts and contamination.

It appears that gas chromatography followed by mass spectrometry is now the most acceptable procedure for the measurement of nitrosamines in food.

The total analytical procedure usually involves an extraction step, followed by distillation; partitioning with solvents; a clean-up step with column or thin-layer chromatography; and, finally, separation, detection, and confirmation. A survey of analytical techniques used in the isolation and detection of nitrosamines has been published by Wassermann¹ and Eisenbrand.¹⁶

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6. ENVIRONMENTAL CONCENTRATIONS AND HUMAN EXPOSURE

6.1 AIR

The presence of N-nitrosamines in ambient air recently has been reported by Bretschneider and Matz,¹ Fine et al.,^{2,3} Pellizzari,⁴ and DuPont.⁵ Bretschneider and Matz,¹ found traces of dimethylnitrosamine and diethylnitrosamine in commercial plants producing dimethyl and diethyl amines. A trace of dimethylnitrosamine was found in one dust sample. Measurements by Fine et al.^{2,3} were made in Baltimore, Maryland; Philadelphia, Pennsylvania; Waltham, Massachusetts; Wilmington, Delaware; Belle, West Virginia; and New York City, New York. Measurements by Pellizzari⁴ were made in Baltimore, Maryland, and Belle, West Virginia, during the same period as the second series of measurements by Fine et al.³ Measurements were made by DuPont⁵ in Belle, West Virginia. Different measurement techniques were used by each of the investigators in Baltimore and Belle. These techniques were described in Section 5. Although the measurement methods have not been thoroughly evaluated, the agreement between the different reported values seems to leave little doubt concerning the presence of N-nitrosamine in some ambient air samples at detectable levels (above $0.01 \mu\text{g}/\text{m}^3$, for example). Stack samples from the dimethylhydrazine plant in Baltimore also contained N-dimethylnitrosamine.

In the initial study by Fine et al.,^{2,3} dimethylnitrosamine concentrations in Baltimore ranging from 0.033 to $0.960 \mu\text{g}/\text{m}^3$ were reported. The reported concentrations in Belle, West Virginia, ranged from 0.014 to $0.051 \mu\text{g}/\text{m}^3$. A trace was found in one sample taken in Philadelphia, but no detectable amount was found in Delaware. Results are shown in Table 6-1.^{2,4,6} Later measurements in Baltimore, Maryland, and in West Virginia by Fine et al.,³ Pellizzari,⁴ and DuPont⁵ revealed higher concentrations in Baltimore and Belle at sampling sites in the immediate vicinity of commercial plants. Values ranged from 1 to $36 \mu\text{g}/\text{m}^3$ at the Food Machinery and Chemical Corporation (FMC) property in Baltimore. These preliminary data are not sufficient for the determination of temporal and spatial distributions.

6.2 WATER

At present, our knowledge about the occurrence of nitrosamines in water supplies is limited to the results of only a few investigations. Shabad and Il'nitskii⁷ reported the occurrence of both nitrosamines and benzo(a)pyrene in a reservoir in Russia. Unfortunately, the details of their investigations are not available at this time.

Sander et al.⁸ in 1974 analyzed tap, river, and waste water purified by a biological treatment plant in West Germany. Although they did not detect any nitrosamines (i.e., ≤ 0.5 parts per billion, ppb) in the samples, they reported that the river water contained 0.6 to 4.3 ppb dichloromethane-extractable nitrogen, 0.18 to 1.1 mg $\text{NO}_2^-/\text{liter}$, and 0.17 ppb each of dimethylamine, diethylamine, and pyrrolidine or piperidine, amines readily converted to nitrosamines. In the purified water samples, they detected 9.3 to 10.4 ppb total nitrogen, 0.64 to 0.83 mg $\text{NO}_2^-/\text{liter}$, and 0 to 2.1 ppb of each of the amines. The tap water, however, contained none of the amines and no NO_2^- or total nitrogen. Dure et al.⁹ investigated the occurrence of nitrosamines in water processed at a Munich, Germany, clarification plant and in sediments from Starnberger Lake and the Main River. In each instance, the nitrosamine level was ≤ 0.1 ppb.

**Table 6-1. NITROSAMINE CONCENTRATIONS IN AMBIENT AIR
MEASURED IN 12 U.S. CITIES OR LOCALITIES^{2-4,6}**

Location	Approx. hours of sampling	Concentration range, $\mu\text{g}/\text{m}^3$ (1- to 2-hr peaks)		
		DMN	Unknown 1 ^a	Unknown 2 ^a
Baltimore, Md., area				
FMC property	54	1 to 36	ND ^b to 0.08	ND ^b to 2.7
Within 0.6-km radius of FMC property	27	0.03 to 7.6	ND ^b to 0.17	0.02 to 0.32
Within 0.6- to 1-km radius of FMC property	30	Trace ^c to 2.0	ND ^b to 0.03	ND ^b to 0.34
Outside 2-km radius of FMC property	14	0.02 to 0.13	0.02 to 0.04	ND ^b to 0.3
Belle, W. Va.	78	ND ^b to 0.98	ND ^b to 0.06	ND ^b to 0.08
So. Charleston and Charleston, W. Va.	30	ND ^b to 0.06	-	-
Nitro, W. Va.	5	ND ^b	-	-
Northern New Jersey	11	ND ^b	-	-
Philadelphia, Pa. ^d	12	ND ^e	ND ^b	ND ^b
Wilmington, Del. ^d	12	ND ^e	ND ^b	ND ^b
Waltham, Mass. ^d	4	ND ^e	ND ^b	ND ^b
New York, N.Y.	10	ND ^b to 0.016	-	-
Denver, Colo. (Eisenhower Tunnel)	1	Trace	-	-
Los Angeles, Calif.	1	ND ^e	-	-
St. Louis, Mo.	1	ND ^e	-	-

^a Unknowns were analyzed only by the TEA technique. Total sampling time for unknowns in Baltimore, Md., was about half that for DMN and in Belle, W. Va., was about one-third that for DMN.

^b ND = not detectable ($< 0.0002 \mu\text{g}/\text{m}^3$).

^c Trace is defined as a detectable but nonquantifiable amount $> 0.0002 \mu\text{g}/\text{m}^3$.

^d Sampled during initial TEA study only.²

^e ND = not detectable ($< 0.1 \mu\text{g}/\text{m}^3$).

In September 1975, Fine et al.² released preliminary data indicating the possible presence of several nitrosamines in New Orleans-area drinking water samples. N-nitrosoatrazine was suggested as the probable identity of one of the nitroso compounds, while the concentration levels of the various nitrosamines were estimated to be ≤ 0.1 ppb.

In late September 1975, the staff of the Water Quality Division of the Health Effects Research Laboratory, EPA, Cincinnati, collected water samples from six rural wells in Washington County, Illinois, and six in Runnels County, Texas. The samples were characterized by both high nitrate levels and coliform counts. The samples were analyzed for nitrosamines by Fine and his associates at Thermo Electron Research Center in Waltham, Massachusetts; in each of the 12 samples the concentration of volatile and nonvolatile nonionic nitrosamines was ≤ 15 parts per trillion (ppt).

Fine et al.³ reported 6000 $\mu\text{g}/\text{liter}$ of dimethylnitrosamine in a rainwater puddle near the unsymmetrical dimethylhydrazine (UDMH) plant in Baltimore. A sample from a drainage ditch on the border of the property contained 5.9 $\mu\text{g}/\text{liter}$, and water samples from the adjacent cove contained 0.26 to 0.94 $\mu\text{g}/\text{liter}$.

For discussion concerning the occurrence of nitrosamines in water, see Section 7.1.3.

6.3 FOOD AND DRUGS

6.3.1 Nitrosamines in Processed Foods

The presence of the carcinogen N-nitrosodimethylamine (DMN) was demonstrated in fish meal as a result of the reaction of sodium nitrite with the secondary amine, dimethylamine.¹⁶ Studies then

followed to investigate the presence of this nitrosamine in human foods treated with sodium nitrite and sodium nitrate, especially in cured meat products.^{11,12} Data reported prior to 1970, however, are open to question because the analytical methodology included nonspecific identification procedures without adequate confirmation of the nitrosamine. The use of the alkali flame-ionization detector for gas-liquid chromatography (GLC), and several other techniques, followed by confirmation with a GLC-mass spectrometric method, has led to the detection and identification of nitrosamines in cured meat products in concentrations as low as 3 to 5 $\mu\text{g/kg}$.

Among the more recent studies are a number of investigations on the presence of nitrosamines in smoked and nitrite-preserved fish.

In a study by Howard et al.,¹³ samples of nitrite-preserved and non-nitrite-preserved smoked chub were analyzed for dimethylnitrosamine (DMN) by means of gas-liquid chromatography (GLC), using a modified thermionic detector. Apparent values of DMN were 1 to 2 ppb (below the GLC quantifiable limit but confirmed by mass spectrometry) in both nitrite-preserved and non-nitrite-preserved samples. These results led the authors to conclude that DMN is not produced in the flesh under the conditions of the commercial processing treatment used.¹³

The possible formation of DMN through nitrosation of amines in smoked chub was studied in aqueous model systems containing methyl amines and nitrites.¹⁴ Reaction conditions employed (pH, reaction time, and temperature) were more severe than those occurring during the commercial processing of nitrite-treated smoked chub. Polarographic analyses indicated that no detectable amounts (1.4×10^{-5} mole) of DMN were produced.¹⁴

Marine fish reportedly have a higher amine content than freshwater fish and, therefore, should have a greater potential for nitrosation reactions. Dimethylnitrosamine was found¹⁵ by GLC and determined by mass spectrometry (MS) in sable samples at the following levels: raw, 4 ppb; smoked, 4 to 9 ppb; smoked nitrite-treated, 8 to 14 ppb; and smoked, nitrite- and nitrate-treated, 23 ppb. Neither raw shad nor salmon contained DMN, but one smoked salmon sample contained 5 ppb DMN and smoked nitrate-treated salmon and shad products contained 10 to 17 ppb.¹⁵

A number of foodstuffs were analyzed for nitrosamines by GLC, using an electron-capture detector, after extraction by neutral steam distillation of samples.¹⁶ Diethylnitrosamine was found at 1.5 $\mu\text{g/kg}$ in Cheshire cheese, fried bacon, raw pig's liver, and stale cod (raw and fried). The cod also contained 1.0 $\mu\text{g/kg}$ DMN and 6.0 $\mu\text{g/kg}$ N-nitrosopyrrolidine (NPyr). Of 25 food samples tested, 16 (7 of which were cod samples) contained NPyr at levels of 1.0 (Norwegian goat's milk and fresh cod) to 11.0 $\mu\text{g/kg}$ (raw pig's liver).¹⁶

In a study by Fong and Chan,¹⁷ samples of marine salt fish from the markets in Hong Kong were analyzed for DMN. Pickling and drying of such fish are done in the open, so that the fish are subject to contamination by bacteria. These samples contained 0.05 to 0.3 ppm DMN as determined by GLC and confirmed in several samples by GLC-MS. Fish prepared with crude salt contained much more DMN than fish prepared with pure sodium chloride. Levels of nitrate were found to be appreciable in the crude salt commonly used to prepare salt fish, amounting to 17, 18, 20, 20, 30, and 40 ppm in six samples of crude salt purchased from local retailers. The nitrite content was no more than 1 ppm in any of these samples. The level of DMN found varied with the species of fish. Salted white herring contained more DMN than salted yellow croaker, and a single batch of pomfret contained no detectable DMN.¹⁷

Fong and Chan further conducted a study on the role of nitrate-reducing bacteria in the formation of nitrosamines.¹⁸ They demonstrated that fish broth inoculated with *Staphylococcus aureus* (10^6 cells/ml) and untreated fish broth (unsterilized, uninoculated) showed an increase in DMN content with time. Sterilized fish broth showed no such change in DMN content (Figure 6-1). Content of DMN was determined by GLC-MS techniques. The authors concluded that the amount of DMN present seems to be dependent on the storage conditions, degree of contamination by nitrate-reducing bacteria, and the amount of precursors present.¹⁸

The effects of processing and storage on sablefish were studied by Gadbois et al.¹⁹ Sablefish was treated with 0 to 1300 ppm nitrite prior to being smoked. The meat was analyzed for the presence of

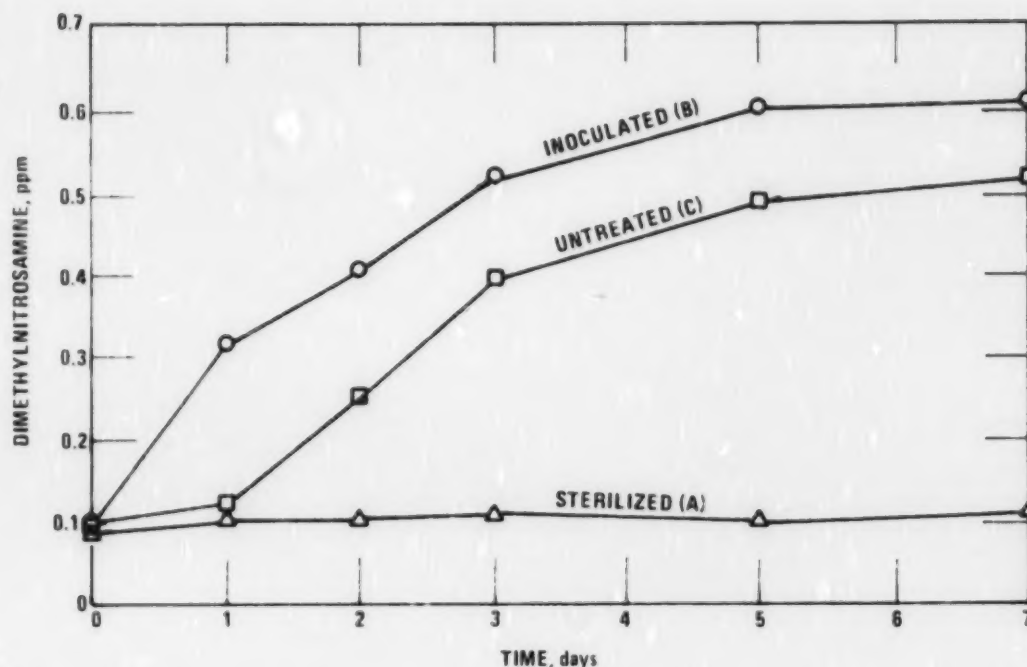


Figure 6-1. Concentration of dimethylnitrosamine in fish broth after incubation at 37°C. (Portion A, sterilized; portion B, inoculated with 10^9 *Staphylococcus aureus* per lot; portion C, untreated.)¹⁸

N-nitroso compounds by gas-liquid chromatography immediately after processing and again after 2 weeks' storage at 40°F. Trace amounts of DMN (< 10 ppb) were detected; nitrite levels in these samples ranged from 0 to 550 ppm. Levels of DMN did not increase with higher nitrite levels, and a slight decrease in DMN concentrations was found after storage at 40°F. The identity of the isolated compound was confirmed by GLC and by GC-MS¹⁹ as the nitramine derivative.

Havery et al.²⁰ surveyed 121 food samples for the presence of N-nitrosamines. N-nitrosopyrrolidine was confirmed in fried bacon at levels up to 139 ppb. Dimethylnitrosamine, N-nitrosopyrrolidine, and N-nitrosopiperidine were also confirmed in spice-cure mixtures at levels ranging from 50 to 2000 ppb.

Included in this survey²⁰ were Icelandic national foods processed under atypical conditions that are theoretically conducive to nitrosamine formation. For example, pieces of shark and skate are coated with coarse salt and buried in the ground, where they remain until a certain stage of fermentation is reached. The putrified fish are hung outdoors for 1 to 3 months and then are eaten without further preparation. Other fish, such as trout and salmon, are pickled in a salt solution containing 0.6 percent nitrite before smoking. These modes of food processing in conjunction with the high concentrations of amines normally found in fish should have provided an ideal environment for nitrosamine formation. Nonetheless, no nitrosamines were found in these samples.²⁰

Conventionally cured hams studied in a number of laboratories around the world have been found free of the nitrosamines for which they were tested, except for an occasional report of a low concentration of DMN.²¹

Only 3 of 41 samples of frankfurters tested were reported to contain DMN;²² unpublished data on other samples of frankfurters showed the absence of this nitrosamine. Other emulsion-type products, however, have been reported to contain variable quantities of DMN and/or N-nitrosopyrrolidine.

dine (NPyr); e.g., Hungarian sausage, mettwurst, salami, dry sausage, thuringer, knockwurst, and thuringer liver sausage. Panelaks et al.²³ surveyed 197 cured meat samples for DMN and found 57 positive, but the identity of the nitrosamine was not confirmed. In an additional 100 samples,²⁴ they found that 29 samples contained DMN, 17 contained NPyr, and 9 contained N-nitrosodiethylamine. Only five of these positive findings, however, were confirmed by mass spectrometry.

The presence of NPyr and N-nitrosopiperidine (NPip) in emulsion-type products was explained with the finding of these two nitrosamines in premixed cures.²⁵ For convenience in handling, cure components — including sodium chloride, sodium nitrite, sodium nitrate, spices, and other ingredients — were premixed to the processor's formulation and stored for several months prior to use. Sodium nitrite can react with components in pepper to form NPip and with unknown paprika components to produce NPyr.²⁶ The source of DMN has not been identified. Regulations have been promulgated now to forbid premixing of nitrite or nitrate salts with other cure ingredients.²⁷

Nitrosamines have also been reported in jellied meat products. DMN and NPyr were found in head cheese, souse, and blood and tongue sausage.²⁸ This class of meat products contains pieces of organ meat or offal previously cured with nitrite or nitrate salts. Methods of preparation, which differ among processors, may include the further addition of nitrite or nitrate salts to the blood. Further studies on the sources of nitrosamines or the mechanism of their formation have not been carried out.

The product of greatest concern, however, is bacon. N-nitrosopyrrolidine has been reported in almost all samples of bacon examined after frying and DMN has been found sporadically. These nitrosamines were not identified in the raw, uncooked bacon; they appear only following the application of heat.²⁹⁻³¹ Consideration has been given to the precursor(s) and the mechanism of formation of NPyr. Proline, which is present in collagen, can be either nitrosated initially to form nitrosoproline (NPro) with decarboxylation to NPyr on heating, or the amino acid can be decarboxylated first to pyrrolidine, and then nitrosated.³² Putrescine, a diamine, can form pyrrolidine by ring closure upon heating. The latter compound can then be nitrosated. The addition of either proline or putrescine and nitrite to ground pork belly resulted in an increase in NPyr formation, although greater amounts were found with proline than with putrescine. Nitrosoproline was identified and the identity confirmed in raw bacon³³ but, because of the difficulties involved in analysis of nonvolatile nitrosamines, the quantitative determination of the concentration present could not be assured.

Studies on the decarboxylation of NPro showed the temperature at which maximum conversion of NPro to NPyr occurs (365°F) approximates that which is recommended for frying or broiling bacon by most processors. Time-temperature studies with bacon fried to the same degree of doneness (medium well done) showed that longer frying times at lower temperatures produced little or no NPyr, while higher frying temperatures led to the formation of the nitrosamine.

N-nitrosopyrrolidine was found in bacon drippings at concentrations as high as, or higher than, those present in the edible portion of fried bacon.^{29,34} Studies with fried, separated, lean and adipose tissue showed that the nitrosamine is produced in the adipose tissue^{29,34} and is not present as a result of the greater solubility in lipid of NPyr produced in the lean. The precursor for NPyr in adipose tissue has not been identified, although it has been suggested that the collagen present in adipose tissue is the source of proline.

In view of the potential carcinogenic nature of NPyr, reduction or elimination of this compound from bacon is desirable. The complete elimination of NPyr should be accomplished by the removal of nitrite or nitrate salts from the cure, although it should be noted that nitric oxides in smoke may be able to react with the bacon amines also. Public health considerations with respect to inhibiting the outgrowth of *Clostridium botulinum* spores, and thus avoiding botulinus poisoning, militate against the complete ban of nitrite in bacon at this time. Reduction of the nitrite concentration, however, could result in lower concentrations of NPyr.³⁵

The prevention or reduction of nitrosamine formation through the use of other compounds must be considered. Sodium ascorbate (or sodium erythorbate), used in processing cured meats to accelerate cure color development, has been found to prevent the formation of experimentally produced DMN in frankfurters³⁶ and NPyr in laboratory-prepared bacon.³⁷ Studies on commercially pro-

duced bacon were sponsored by the American Meat Institute in cooperation with the Food and Drug Administration and Department of Agriculture. Results with commercial batches of product confirmed the reduction of NPyr concentration by ascorbate; but, for reasons still unknown, the nitrosamine was not completely eliminated. Studies are in progress to determine the effects of decreasing concentrations of nitrite and increasing concentrations of ascorbate.

It has been reported that phenols present in the smoke used in processing react with nitrite,^{38,39} thus potentially reducing the concentration of nitrite available for nitrosation of the amines.

The Expert Panel on Nitrite and Nitrosamines, appointed by the Secretary of Agriculture, has recommended the complete elimination of nitrate from cured meat products (except, temporarily, in dry-cured and fermented products) and the reduction of residual nitrite to various concentrations that would depend on the class of meat product.

Since nitrite is a chemical and possibly a physiological precursor of nitrosamines, and since nitrate is easily reduced to nitrite under physiological conditions, the levels of both nitrate and nitrite entering the digestive tract are pertinent to any assessment of exposure to nitrosamine precursors. White⁴⁰ calculated the average daily ingestion by a U.S. resident of nitrate and nitrite. Rather than calculate possible ingestion from hypothetical meals, White based his estimates on per capita consumption and production statistics and on recent nitrite data for 34 vegetables. His estimates are presented in Table 6-2.⁴⁰

Table 6-2. ESTIMATED AVERAGE DAILY INGESTION OF NITRATE AND NITRITE BY U.S. RESIDENT⁴⁰

Item	Nitrate		Nitrite	
	mg	%	mg	%
Vegetables	86.1	81.2	0.20	1.6
Fruits, juices	1.4	1.3	0.00	0.0
Milk and products	0.2	0.2	0.00	0.0
Bread	2.0	1.9	0.02	0.2
Water	0.7	0.7	0.00	0.0
Cured meats	15.6	14.7	3.92	30.7
Saliva	30.0 ^a	-	8.62	67.5
Total	106.0	100	12.76	100

^a Not included in total; see text.

According to these data, four-fifths of dietary nitrate originates with vegetables and only one-sixth from cured meats. Other sources are insignificant. Two-thirds of the nitrite entering the stomach originates in saliva, and slightly less than one-third comes from cured meats. White assumes salivary nitrate to come from food and does not include it in the total nitrate ingested. He assumes salivary nitrite to originate in the mouth and hence includes it in intake.⁴⁰

White's data do not represent actual ingestion but are averages calculated from available data.

6.3.2 Nitrosamines in Plants and Fruits

Nitrosamines in the range of 0.4 to 30 $\mu\text{g/kg}$ have been found in some samples of mushrooms.⁴¹ Hedler and Marquardt⁴² found diethylnitrosamine in samples of wheat plants, grain, and flour; but the method was not quantitative and was not confirmed. Thewlis⁴³ was unable to find diethylnitrosamine in wheat flour. Kroller⁴⁴ analyzed 30 samples of wheat flours and was unable to detect nitrosodiethylamine at levels greater than 0.01 mg/kg in more than one of these samples. DuPlessis et al.⁴⁵ found dimethylnitrosamine in the fruit of a plant (*Solanum incanum*) in South Africa. Cycad nuts are known to be toxic, and Laquer et al.⁴⁶ reported that rats fed the nuts developed liver and kidney tumors. Keybets et al.⁴⁷ examined spinach in which nitrate-reducing bacteria produced a high nitrite

level, but did not detect nitrosamines. They concluded that the pH was too high and the concentration of secondary amines too low to allow the formation of nitrosamines. Tobacco contains several secondary amines and up to 1000 ppm of oxides of nitrogen; hence, the possibility for the formation of nitrosamines exists. Rhoades and Johnson⁴⁸ recently identified dimethylnitrosamine in tobacco smoke condensation. They also found that growing conditions greatly affect the amount of DMN found in tobacco.

6.3.3 Nitrosamines in Drugs, Pesticides, and Natural Products

Secondary and tertiary amino compounds used as drugs, pesticides, and herbicides, and some natural products such as spermine and spermidine, have been shown to react with nitrite to form N-nitroso compounds.

Lijinsky and his colleagues have pointed out that many drugs and pesticides contain tertiary amino groups⁴⁹⁻⁵³ and may therefore be expected to undergo nitrosation in the body. Examples of drugs that react with nitrite *in vitro* to give varying yields of carcinogenic nitrosamines are oxytetracycline (an antibiotic) and aminopyrine (an analgesic), which yield dimethylnitrosamine; disulphiram (an antialcoholic drug) and nikethamide (a respiratory stimulant), which yield diethylnitrosamine; and tolazamide (an oral hypoglycemic drug), which gives nitrosohexamethyleneimine.

Among commonly used pesticides, several are esters of N-methyl-carbamic acid and therefore are analogs of N-methylurethane. They react with nitrous acid to form N-nitroso derivatives; for example, nitrosocarbaryl.⁵⁴ A study of the *in vitro* nitrosation of carbaryl under simulated gastric conditions showed that 10^{-3} M carbaryl reacted with a fivefold molar excess of nitrite to form 1.7 percent of theoretical yield of N-nitrosocarbaryl after 60 minutes.⁵⁵ Such derivatives could be formed in the environment or *in vivo* from nitrite and residues of the carbamates in crops; for example, nitrosocarbaryl, both carcinogenic and mutagenic. This is a less potent carcinogen than nitrosomethylurethane but a more potent mutagen.

Widely used agricultural chemicals such as zinc dimethyldithiocarbamate (ziram); 1-naphthyl-N-methylcarbamate (propoxur); N-(2-benzothiazolyl)-N'-methylurea (benzthiazuran); 2-chloro-4,6-bisethylamino-1,3,5-triazine (simazin); and 2-chloro-4-ethylamine-6-isopropylamino-1,3,5-triazine (atrazine) have been found to nitrosate easily.⁵⁶ Dimethylnitrosamine (DMN) has been shown⁵⁷ to be the product of the *in vitro* and *in vivo* (in the rat) reactions of ziram with sodium nitrite. The *in vitro* reaction of ferbam (iron dimethyldithiocarbamate) with sodium nitrite also produced DMN. Atrazine has been shown⁵⁸ to react *in vitro* with nitrite (at pH 2.0) about two orders of magnitude faster than dimethylamine and about three orders of magnitude faster than carbaryl.

A recent investigation by Fine and Ross⁵⁹ of six commercial weed killers revealed nitrosamines at levels ranging from 0.3 mg DMN/liter in a home lawn-care product to 640 mg DMN/liter in an industrial herbicide. Dipro, ylnitrosamine (DPN) was found at 154 mg/liter in Treflan, a major agricultural herbicide. These findings are summarized in Table 6-3.⁵⁹ The DMN detected in Trysben 200 is apparently a product of the reaction between dimethylamine, a Trysben 200 component, and sodium nitrite used as a corrosion inhibitor in 1-gallon cans of the herbicide. (The DMN is 0.1 percent by weight; the minimum level regulated by the Occupational Safety and Health Administration is 1 percent by weight.)⁶⁰

Because the agricultural herbicide, Treflan, is used on food crops, the investigators sampled air and irrigation water from tomato fields in the Sacramento Valley, California, before, during, and after application of Treflan in May 1976.⁵⁹ No DPN was found in any of the ambient air samples (detection limit = 1 ng/m³) or in any of the water samples (detection limit = 20 ng/liter). Preliminary analyses of tomatoes picked prematurely (September is harvest-time) showed no detectable amount of DPN. Tests on Treflan by its manufacturer have not shown the product to be carcinogenic. Fine and Ross postulated that the incomplete removal of nitric acid used in the synthesis of the product could result in nitrosation of dimethylamine, also used in the manufacturing process.⁵⁹

Table 6-3. N-NITROSAMINES IN WEED KILLERS⁵⁹

Product	Use	N-nitrosamine	Concn., mg/liter	Active ingredients
Black Leaf Weed Killer	Home lawn care	DMN	— ^a	12.72% 2,4-Dichloro- phenoxyacetic acid as dimethylamine salt 4.07% 2,4,5-Trichloro- phenoxypropionic acid as dimethylamine salt
Sears Broad Leaf Weed	Home lawn care	DMN	— ^a	12.00% 2,4-Dichloro- phenoxyacetic acid as dimethylamine salt 2.40% 3,6-Dichloro-o- anisic acid as dimethyl- amine salt
Unico Turf Treeter T	Home lawn care	DMN	0.3	10.59% 2-(2-Methyl-4- chlorophenoxy) propionic acid as dimethylamine salt 3.23% 2,4-Dichlorophenoxy- acetic acid as dimethyl- amine salt 1.28% 3,6-Dichloro-o- anisic acid as dimethyl- amine salt
Du Pont Trysben 200 Weed Killer	Industrial herbicide	DMN	187-195	26.10% 2,3,6-Trichloro- benzoic acid and related polychlorobenzoic acids as dimethylamine salt
Benzac 1281	Industrial herbicide	DMN	640	26.10% 2,3,6-Trichloro- benzoic acid and related polychlorobenzoic acids as dimethylamine salt
Treflan E.C.	Agricultural herbicide	DPN	154	44.50% α,α,α -Trifluoro- 2,6-dinitro-N,N-dipropyl- p-toluidine

^a Less than 0.05 mg/liter.

6.4 RELATIVE EXPOSURE

Nitroso compounds or their precursors are widely distributed in the environment, resulting from both natural processes and human activities. Exposure may be via inhalation, ingestion, and, for the human species, smoking of tobacco. Exposure may be direct by these routes, or nitrosamines (as well as other nitroso compounds) may be formed *in vivo* from nitrogen and amine compounds. Therefore, the potential for exposure to very low levels of carcinogenic nitroso compounds is ever-present.

It is not possible at this time to provide a meaningful estimate of either total or relative exposure via each route of intake. Nitrosamines have been found in a variety of foodstuffs in varying concentra-

tions, but many reported values are doubtful because of sampling and analytical problems. Nitrosamines have been found in the ambient air in the vicinity of a man-made source at concentrations of a few parts per billion. Theoretical considerations suggest that nitrosamines might be found in polluted ambient atmospheres (possibly in the parts-per-trillion range) and therefore may be a potential source of direct exposure via inhalation.

There is a substantial body of evidence indicating that nitrosamines (and other carcinogenic nitroso compounds) can be formed *in vivo* from nitrates and amines (precursors). Exposure to these precursors again may be via ingestion, inhalation, and smoking. It has been estimated⁶¹ that a single meal may contain as much as 100 mg of secondary amines, with an average daily intake of 20 to 30 mg. The average nitrite production in human saliva has been estimated at 6 to 10 mg/day. A single cigarette may contain 0.5 mg nitrite.

The intake of nitrate nitrogen from drinking water could be as much as 100 mg/day in dry areas where farm wells contain over 50 mg/liter of nitrate nitrogen. (The EPA standard is 10 mg/liter nitrate nitrogen.)

Using a standard respiratory volume per day, and assuming an exposure to nitrate concentrations of 3 to 5 $\mu\text{g}/\text{m}^3$ for 12 hours per day, the calculated inhaled nitrate would be 21.5 μg per day. Peak atmospheric nitrate levels in some local areas may exceed 100 $\mu\text{g}/\text{m}^3$. Kant⁶² found that nitrosamines were formed in rat lung homogenates after a gas mixture containing 15 percent nitrogen dioxide was bubbled through the homogenates. (It is known that nitrites are produced when nitrogen dioxide is bubbled through water.) However, when rats inhaled an atmosphere containing 0.01 to 0.5 mg nitrogen oxides per liter, no nitrogen oxide compounds were found in the lung tissue. No data are available regarding *in vivo* formation of nitrosamines in humans as a result of inhalation of nitrogen dioxide. Further, no estimates of amine concentrations in the atmosphere are available.

It is apparent that under normal living conditions there is a potential for the formation of nitrosamines *in vivo*, though meaningful estimates of the total burden cannot be made. It seems evident, however, that exposure to precursors via inhalation is relatively small compared to that from food, water, and smoking.

Again, a reliable daily dietary intake of nitrosamines is not available; but, based on measured concentrations in foodstuffs and water, the direct daily intake is not likely to exceed a few micrograms per day. In the vicinity of a nitrosamine emission source, reported values indicate that the average daily intake via inhalation may reach a few micrograms per day. Assuming 20 m^3 of air are breathed per day, the daily intake via inhalation would therefore be comparable to that from ingestion. For populations not living in the vicinity of emission sources, the daily intake via inhalation would likely be less than 1 $\mu\text{g}/\text{day}$.

6.5 POPULATIONS AT RISK

Our present knowledge does not permit us to estimate the risk of tumor induction in the human population resulting from exposure to nitrosamines. This would require much more definitive information than is now available concerning dose-response relationships in humans and exposure patterns over time. Though evidence of carcinogenic potential from animal experiments seems conclusive, there is at present no definitive information that nitroso compounds have caused cancer in humans under any conditions.

With our present state of knowledge, risk of exposure can be discussed only in a qualitative manner. Data indicate rather clearly, however, that the entire human population is continuously exposed to very low levels (ppb or ppt) of nitroso compounds via ingestion. Exposure to nitrosamines via drinking water does not appear to be as widespread as via food, and is probably very small. Detectable quantities have been found in only a few water supplies.

Nitrosamines have been measured in ambient air in the vicinity of emission sources in the ppb range. In addition, chamber experiments and theoretical considerations suggest that nitrosamines may be formed in polluted air. Concentrations in the ppt or the low ppb range may occur, at least under con-

ditions favorable to nitrosamine formation, in all major industrial areas. Therefore, there is a potential risk of direct exposure via inhalation to low concentrations among major segments of the population.

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7. SOURCES

7.1 NATURAL OCCURRENCE

Nitrates, nitrites, and amines, which are precursors in the formation of nitrosamines,¹ are chemical compounds that occur naturally in food, water, soil, and air. Unlike many man-made chemical compounds currently causing environmental problems, the above-named chemical compounds have probably been in existence throughout the evolutionary history of man.

Nitrites are in themselves toxic to man, other animals, and plants. However, the nitrosamines that may be formed in soil, water, air, food, and in the gastrointestinal tract through the chemical and microbial nitrosation of secondary and tertiary amines are the greater hazard to man.¹

7.1.1 Formation of Nitrogen Compounds in the Nitrogen Cycle

Natural occurrence of nitrates, nitrites, and secondary and tertiary amines is the result of their formation during the nitrogen cycle. Except for man-made fertilizers, all nitrogen necessary for the existence of terrestrial or aquatic organisms becomes available through the nitrogen cycle. Nitrogen in the form of nitrates or ammonia is taken up by plants and incorporated into proteins or nucleic acids. When plants are eaten by animals, the proteins and nucleic acids become available to the animals for use in forming their own nitrogen compounds. In the environment, through the breakdown of animal excreta and the decay of dead plant and animal tissues by microorganisms, complex nitrogen-containing compounds are ultimately converted into ammonia. Should a portion of the decay process occur without free oxygen, amines may be formed.² These transformations of nitrogen are mediated almost entirely by microorganisms^{2,7} and involve organic, inorganic, and volatile compounds. In general outline, the nitrogen cycle is identical in terrestrial and aquatic habitats; only the microorganisms that mediate the various transformations are different.^{3,6} Though termed a cycle, the transformations of nitrogen occur simultaneously and involve its being shuttled back and forth into different forms.²

The largest source of elemental nitrogen is the atmosphere.⁸⁻¹⁰ For nitrogen to become available for use by plants and animals, it must be transformed or fixed into organic nitrogen. Small amounts of nitrogen are fixed in the atmosphere and come down to earth in rainwater as ammonia and nitrate salts,^{2,6-8} but biological fixation is the principal way in which nitrogen becomes available to life on earth.

7.1.1.1 Biological Nitrogen Fixation—Biological fixation results in the transformation of nitrogen gas into nongaseous nitrogen compounds. The transformation is carried out by a variety of microorganisms, some of which live symbiotically in the root nodules of plants (chiefly legumes) while others are free-living in the soil. The process may be accomplished under aerobic or anaerobic conditions.

7.1.1.2 Organic Nitrogen Formation (Assimilation)—Fixed nitrogen, as either ammonia or nitrates, is assimilated by microorganisms and plants and converted into plant protein, nucleic acids, and various other organic complexes. Plants are eaten by animals and animals by other animals. Nitrogen is immobilized in the many nitrogen-containing compounds until the organisms die or, in the case of animals, until certain products are excreted.

7.1.1.3 Mineralization (Ammonification)—In transformation by mineralization, or ammonification, a portion of the large reservoir of organic complexes in the soil is decomposed and converted into inorganic ammonia. Microbial action results in the breakdown of protein, polypeptides, nucleo-

proteins, nucleic acids, and aromatic compounds. From the degradation of protein-rich materials, if the transformation proceeds anaerobically, the final products can be ammonia and amines.²

The ammonia formed during mineralization may be assimilated by aquatic plants, terrestrial plants, or by microorganisms; may be bound by clay particles in the soil; may escape into the air; or may be converted by microorganisms into nitrates in the nitrification process. Under anaerobic conditions, ammonia accumulates because nitrification cannot occur.

7.1.1.4 Nitrification—Nitrification is the microbial conversion of ammonia to nitrites and nitrates. Nitrification occurs in the soil, in manure piles, during sewage processing and in marine environments.² The first step in the conversion is the oxidation of ammonia to nitrite ion by *Nitrosomonas* bacteria. These bacteria use ammonia as their sole source of energy. Nitrite in turn is rapidly oxidized by *Nitrobacter* organisms usually found in association with *Nitrosomonas*. In some cultures, nitrite may accumulate if the *Nitrobacter* cannot oxidize all the nitrites produced by *Nitrosomonas*.^{2,5,7} Nitrite is usually converted to nitrate so rapidly that it seldom accumulates in the soil; in alkaline soils, however, nitrite accumulation may occur if levels of ammonium are high.

Nitrates, whether added to the soil in fertilizers or formed by nitrification, may be assimilated by plants; washed downward through the soil into ground water or through surface runoff into streams, rivers and oceans; may be transformed into atmospheric nitrogen during microbial denitrification; or may be reduced by microorganisms to ammonia.

7.1.1.5 Nitrate Reduction—Microorganisms can utilize nitrates to build organic nitrogen compounds just as plants do, or they can utilize some or all of the nitrate for its oxygen content if local conditions are anaerobic. The second case is denitrification (see below). Incomplete reduction may result in an accumulation of nitrite.

7.1.1.6 Denitrification—When certain bacteria are deprived of oxygen, they will turn to nitrates, NO_3^- , as a source of oxygen. The first step is the reduction of nitrate to nitrite. Many bacteria are capable of reducing nitrite to N_2 or N_2O gases, again under local anaerobic conditions. If the two types of reduction are not in balance, nitrites may accumulate in the medium. Further, nitrites may accumulate in alkaline soils.² Only local anaerobic conditions are necessary for denitrification and significant denitrification may occur in the center of aerated flocs of bacteria or at the base of slime layers. The ratio of N_2 to N_2O produced probably depends on pH, temperature, and other environmental factors.

Figure 7-1 is a diagrammatic presentation of the movement of nitrogen in the biosphere. The transformation and movement of nitrogen as explained in the foregoing pages relate to the biogeochemical circulation of nitrogen. The circulation of nitrogen is a long-term process. Turnover times for the three largest "pools" of nitrogen are 3×10^5 years for atmospheric nitrogen; 2500 years for nitrogen in the seas when nitrates and organic compounds are counted together; and less than 1 year for nitrates and nitrites in the soil.⁵ A more detailed account of the distribution and annual transfer rates is shown in Table 7-1. Because the nitrogen cycle is so complex and has so many exchange rates, it is difficult to estimate with a great degree of accuracy. Table 7-1 lists the estimates with probable errors. Thus the transfer rates can be estimated only within broad limits.⁶⁻¹¹ The only two quantities of nitrogen known with any degree of accuracy are the amount of nitrogen in the atmosphere and the rate of industrial fixation.

7.1.2 Nitrogen Compounds In Soil

The levels of nitrate and nitrite nitrogen in the soil are in a constant state of flux. Nitrites exist in the soil for only short periods of time. Under normal conditions, nitrite is rapidly converted to nitrates, nitrous oxide, and nitrogen; or undergoes spontaneous decomposition to nitric oxide.^{2,3,5} Nitrite does accumulate in alkaline soils when ammonium levels are high, however.² The accumulation results because the nitrite formed is not metabolized further to nitrate. The effect on nitrite accumulation of the interaction of alkalinity and ammonium is illustrated in Table 7-2. The accumulation of nitrite in calcareous soils is proportional to the rate of ammonium addition; under constant nitrogen fertilization the effect increases as the hydrogen ion concentration decreases.² With decrease in pH

Table 7-1. BUDGET FOR THE NITROGEN CYCLE^{a,11}

Item	Land		Sea		Atmosphere	
	Million MT/yr	Error, ^a %	Million MT/yr	Error, %	Million MT/yr	Error, %
Input						
Biological nitrogen fixation	—	—	10	50	—	—
Symbiotic ³¹	14	25	—	—	—	—
Nonsymbiotic ³¹	30	50	—	—	—	—
Atmospheric nitrogen fixation ³¹	4	100	4	100	—	—
Industrially fixed nitrogen fertilizer ³¹	30	5	—	—	—	—
N-oxides from combustion	14	25	6	25	20	25
Return of volatile nitrogen compounds in rain	?	—	?	—	—	—
River influx ³¹	—	—	30	50	—	—
N ₂ from biological denitrification ³¹	—	—	—	—	83	100
Natural NO ₂	—	—	—	—	?	—
Volatilization (HN ₃)	—	—	—	—	?	—
Total	> 92		50		> 103	
Storage						
Plants ³¹	12,000	30	800	50	—	—
Animals ³¹	200	30	170	50	—	—
Dead organic matter ³¹	760,000	50	900,000	100	—	—
Inorganic nitrogen ³¹	140,000	50	100,000	50	—	—
Dissolved nitrogen ³¹	—	—	20,000,000	10	—	—
Nitrogen gas ³¹	—	—	—	—	3,800,000,000	3
NO + NH ₄ ²⁵	—	—	—	—	< 1	50
NH ₃ + NH ₄ ¹⁷	—	—	—	—	12	50
N ₂ O ³³	—	—	—	—	1,000	50
Total	912,200		21,000,970		3,800,001,013	
Loss						
Denitrification ³¹	43	—	40	100	—	—
Volatilization	?	—	?	—	—	—
River runoff ^{31, 32} (includes enrichment from fertilizers)	30	50	—	—	—	—
Sedimentation ³¹	—	—	0.2	50	—	—
N ₂ in all fixation processes	—	—	—	—	92	50
NH ₃ in rain ¹⁷	—	—	—	—	< 40	50
NO ₂ in rain	—	—	—	—	?	—
N ₂ O in rain	—	—	—	—	?	—
Total	73		40.2		> 132	

^a The error columns list plus-or-minus probable errors as a percentage of the estimate

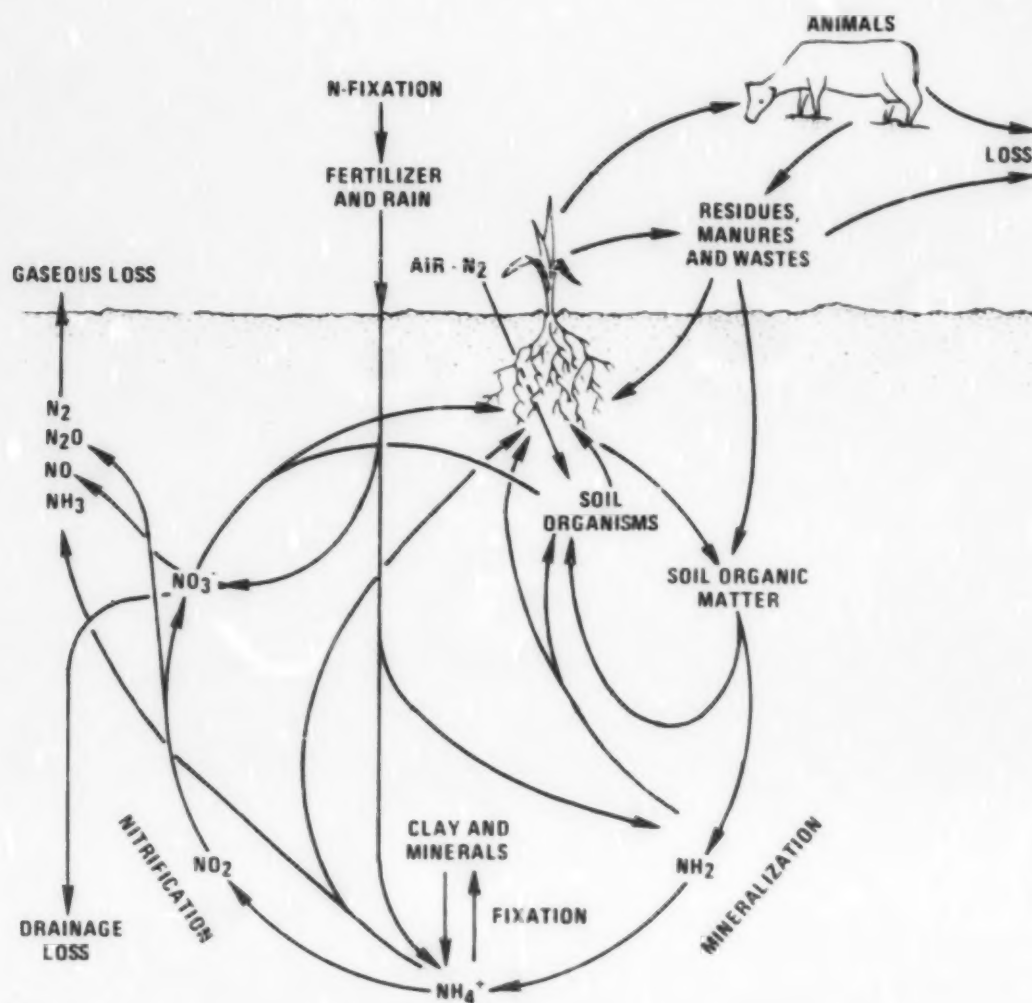


Figure 7-1. Main portions of the nitrogen cycle. Additions of chemical fertilizer make up an increasingly important source of this element.⁷

or ammonium levels, the suppression of nitrate formation is relieved. The sensitivity to ammonium salts exhibited by microorganisms (*Nitrobacter* group) that transform nitrite to nitrate appears to be the factor in nitrite buildup.

Through microbiological action, nitrates in the soil are constantly being formed and also being lost. Losses can be caused by assimilation by higher plants or microorganisms, loss through drainage and runoff water, or loss through transformation into gaseous nitrogen compounds. A measure of nitrate concentrations in soil is, therefore, only an indication of short-term concentrations.^{2,3,5,7} To be available to plants and microorganisms, nitrates must be in the soil solution. Bowen¹² lists the total nitrogen content of the soil as ranging from 200 to 2500 ppm, while the nitrate-nitrogen in soil solu-

Table 7-2. EFFECT OF LIME AND NITROGEN ON NITRITE ACCUMULATION²

Treatment	Nitrate nitrogen, ppm			
	Soil 1	Soil 2	Soil 3	Soil 4
None	0	0	0	0
Calcium carbonate + calcium sulfate [CaCO ₃ + CaSO ₄], 1%	54	59	5	5
Ammonium sulfate [(NH ₄) ₂ SO ₄], 500 ppm N	3	0	0	0
Calcium carbonate + ammonium sulfate [CaCO ₃ + (NH ₄) ₂ SO ₄]	255	218	260	310

tion is considered to range from 2 to 800 ppm. High soil nitrate levels have been found chiefly in areas of low rainfall as a result of nitrification and absence of leaching through runoff.

As mentioned in the previous section, amines are formed in soil during the degradation of protein-rich organic matter, as in crop residues and in green and farm manures. Further, dimethylamine itself is found in urine and feces¹³ and would be present in manures. Humus is also believed to contain amino combinations.^{2,7}

Ayanaba et al.¹⁴ have noted the formation of dimethylamine in soil from trimethylamine. It forms readily at pH values of 5.8 and 6.5 but slowly at a soil pH of 3.8.

Ayanaba et al.¹⁵ have shown that microorganisms in soil may participate in the formation of nitrosamines, such as dimethylnitrosamine (DMN), in one of three ways: (1) by converting tertiary amines or other nitrogenous compounds to secondary amines; (2) by forming nitrite through the reduction of nitrate or oxidation of ammonium, the latter leading to appreciable nitrite accumulation in alkaline environments; and (3) by causing an enzymatic reaction between nitrite and the secondary amine.

Mills¹⁶ has noted the formation of nitrosamines in soil and water at neutral pH's in the presence of colloidal material. He also reported that the formation of nitrosamines in soil need not be mediated by microbial action.

Tate and Alexander¹⁷ reported the persistence of nitrosamines when Williamson silt loam was amended with N-nitrosodimethylamine, N-nitrosodiethylamine and N-nitrosodi-n-propylamine at three different levels (Figure 7-2). However, in the event of rainfall, nitrosamines may be rapidly leached from the soil.¹⁸

The precursors of nitrosamine formation are unquestionably present in soil, but the extent to which nitrosamines are actually formed in the soil and therefore represent a potential health hazard to man is not known.

7.1.3 Nitrogen Compounds in Aquatic Habitats

Aquatic habitats contain the precursors of nitrosamines, just as soil does. In the discussion on the natural occurrence of nitrosamines, mention was made that the nitrogen cycle in terrestrial and aquatic habitats is the same in general outline, with only the microorganisms that mediate the transformations being different.^{3,6} Nitrates, nitrites, and amines are all found in aquatic habitats.^{16,19,20} Nitrates are not only formed in the nitrogen cycle, but are frequently present at high levels in runoff waters in agricultural areas. A study made in Illinois indicates that nitrate concentrations in the Kaskaskia and Sangamon Rivers peaked in May and June, the time of heavy chemical fertilizer application.²¹ This was in contrast to nitrate levels in the Skillet Fork River which drains an area not heavily farmed. The Skillet Fork showed no May-June peak (Figure 7-3). Another study made in the

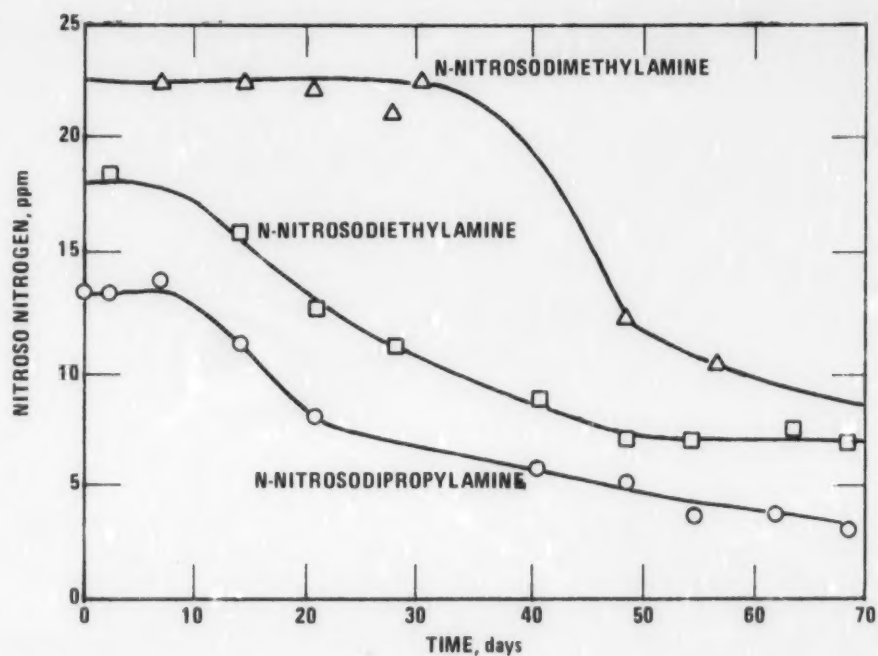


Figure 7-2. Disappearance of three nitrosamines from Williamson silt loam.¹⁸

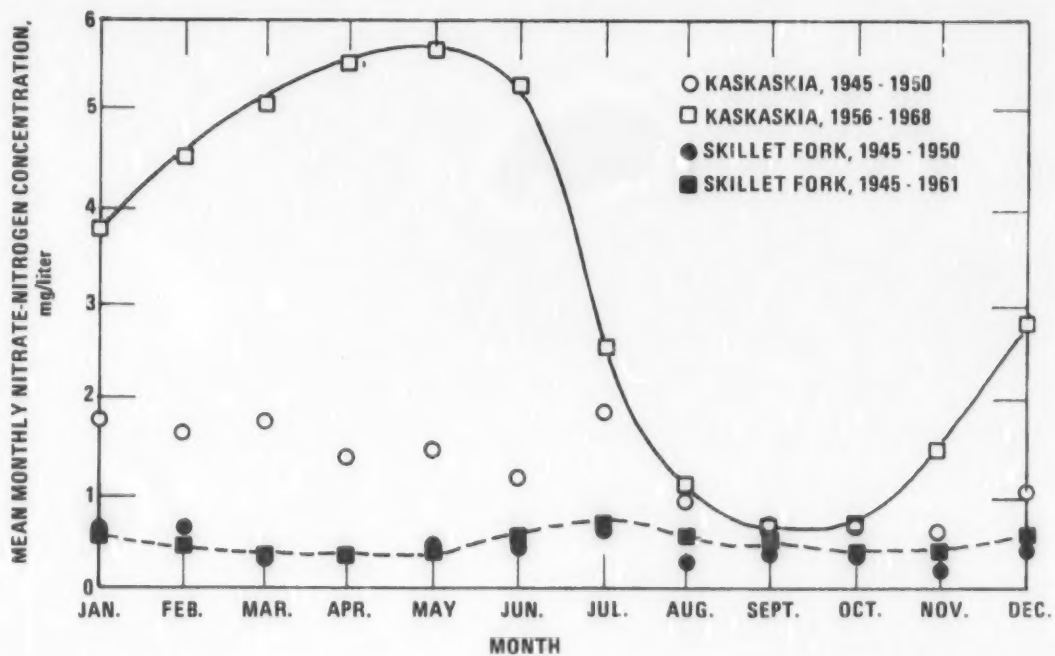


Figure 7-3. Nitrate concentrations in the Kaskaskia and Sangamon Rivers.²²

area of the Sangamon River watershed using nitrogen-15 led to the estimate that at the time of peak nitrate concentration in the spring a minimum of 55 to 60 percent of nitrate-nitrogen found in surface waters of the watershed was from fertilizer nitrogen.²²

Precipitation, sewage, and nitrogenous wastes from farms and packing houses, as well as the use of organic chemicals such as pesticides, are other sources of nitrogen that can lead to increases of nitrates, nitrites, and amines in water.²¹

Ground water receives nitrates from many of the same sources as surface waters;²¹⁻²³ fertilizers, runoff, and percolation from feedlots are among the main contributors. Nitrites are infrequently found in low concentrations because they can be readily oxidized to nitrates.²³

Specific concentrations of nitrates in streams or particularly in lake waters will depend on the sources of the nitrates. The concentration of any nitrogen compound in water is the net result of the rates of nitrogen immobilization (assimilation by aquatic organisms), mineralization (decomposition of organic complexes), nitrification (formation of nitrates), and denitrification (production of gaseous nitrogen).⁸ These levels vary during the year with the NO_3 -nitrogen and NO_2 -nitrogen levels in lakes being high in the spring and minimal during middle to late summer. The NH_4 -nitrogen levels, on the other hand, are usually highest in surface waters in the fall and highest in deep waters during summer.²⁰ The levels are influenced by biological utilization and/or denitrification.²⁰ The effects of biological activity may also be seen in many lakes that exhibit a NO_3 -nitrogen distribution pattern in which levels are low in surface and bottom waters and at a maximum in intermediate depths. In surface waters, the nitrate is immobilized, and in bottom water, denitrified.²⁰ Not all aquatic plants utilize NO_3 -nitrogen. Some take up NH_4 -nitrogen preferentially over NO_3 -nitrogen. The "bloom" of aquatic organisms has been associated chiefly with high NO_3 -nitrogen levels.

Ayanaba, Alexander, and their coworkers^{13-15,22} have demonstrated that nitrosamines can be formed in aquatic environments. Dimethylnitrosamine (DMN) was formed in lake water upon the addition of dimethylamine (DMA) and nitrite (NO_2^-), DMA and nitrate (NO_3^-), trimethylamine and NO_2^- , or trimethylamine and NO_3^- . Mills¹⁶ noted the formation of DMN in sterile and nonsterile lake water and sewage, just as had occurred in soil. On the basis of these studies, he concluded, first, that microorganisms are not necessary to bring about the combination of DMA and NO_2^- to form DMN even at pH values near neutral; second, that the chemical nitrosation reaction is promoted by conditions of low pH and high concentrations of organic matter; and, third, that in this situation the role of microorganisms in the formation of DMN is an indirect one and includes the formation of secondary amine precursors, formation of NO_2^- , and modification of the environment through the production of organic matter and pH alteration. Secondary amines are widespread in nature, but their concentration in soil and water is usually low. An accumulation of secondary amines in water could result in spontaneous nitrosation. The major requirement for nitrosation in water and soil is probably the simultaneous presence of precursors.¹⁶

7.1.4 Nitrogen Compounds in Plants

Nitrosamines are not commonly found in plants, but the precursors necessary for their formation are. Dimethylnitrosamines, however, have been detected in a member of the tobacco family, *Solanum incanum*, used as food by the Transkeian Bantu.²⁶

Secondary amines and nitrites are the chief precursors needed for the formation of nitrosamines. Secondary amines such as DMA, diethylamine, methylethylamine, and methylpropylamine have been found in tobacco plants. Algae have been found to contain many amines, including DMA, trimethylamine, and other methylamines.¹⁶ Nitrites are found only in small concentrations in plant tissues.²⁷ The sources of nitrites in food are nitrates in plants which become reduced after harvest.^{16,21,27} The nitrate levels in plants are of interest, therefore, in determining potential nitrite levels.

In most plants, nitrate is reduced to ammonia and then used in the synthesis of protein and other organic compounds.²⁸ Accumulation of nitrate in plants may indicate that the rate of assimilation has not kept up with the rate of uptake, but the accumulation of nitrate in such cases is only temporary and diminishes as the plant ages.²⁷ The stage of development and a variety of environmental

factors influence the nitrate content, so that variation from plant to plant, as well as within species, genus, or family, may be very great.²⁷ Plants that normally have low levels of nitrate may under certain conditions accumulate it to very high levels. Perennial forage grasses have been found to be low in nitrate in many tests, but have been observed to accumulate nitrate in other cases.

The age of plants, plant variety, site of growth of some plant species, soil type, and environmental factors such as moisture, temperature, pH, and the nitrate concentration in the soil all are important in determining the plant nitrate concentration.^{27,28}

High nitrate levels in plants are associated with rapid assimilation and low rates of reduction. The nitrate content of oats, corn, sugar beets, and *Pennisetum* (Napiergrass) are given in Tables 7-3 through 7-6.^{27,28}

The plant families most often considered to be accumulators of nitrates are: Amaranthaceae (Amaranth), Chenopodiaceae (Goosefoot), Cruciferae (Mustard), Asteraceae (Aster), Poaceae (Grass), and Solanaceae (Nightshade). No single family has been thoroughly sampled for nitrate accumulation.²⁷ Of the families listed, the Mustard, Grass, and Nightshade families contain the largest number of edible plants.

Table 7-3. NITRATE-NITROGEN CONTENT, AS PERCENTAGE OF DRY MATTER, OF OAT VARIETIES GROWN FOR HAY UNDER IRRIGATION AND FERTILIZATION (LARAMIE) AND UNDER DRY LAND CONDITIONS (ARCHER) IN WYOMING¹¹

Variety	Stages ^a								Overall mean
	Laramie				Archer				
	1	2	3	4	1	2	3	4	
Improved Garry	1.22	0.87	0.78	0.65	0.40	0.41	0.20	0.18	0.59
Swedish Select	1.36	1.06	0.84	0.87	1.00	0.64	0.42	0.19	0.80
12-Variety average	1.32	1.00	0.81	0.00	0.58	0.48	0.33	0.20	0.67

^a Stages: 1 — 25% of heads flowering; 2 — 50% of heads in milk; 3 — 50% of heads in soft dough; 4 — 50% of heads in hard dough.

Table 7-4. DISTRIBUTION OF NITRATE-NITROGEN IN DENT CORN PLANTS, EXPRESSED AS PERCENTAGE OF AIR-DRY WEIGHT¹¹

Nodal position from root to tassel	Nitrate-nitrogen, % of air-dry wt						
	Leaf lamina	Leaf midrib	Leaf sheath	Internode	Shank	Ear (fertile)	Tassel
1	0.049	0.081	0.126	0.293	—	—	—
2	0.034	0.063	0.060	0.269	0.085	—	—
3	0.024	0.052	0.060	0.202	0.046	—	—
4	0.014	0.048	0.043	0.139	0.049	—	—
5	0.007	0.031	0.032	0.120	0.027	0.008	—
6	0.007	0.031	0.024	0.097	0.020	0.011	—
7	0.006	0.001	0.021	0.092	0.015	0.010	—
8	0.007	0.004	0.012	0.092	—	—	—
9	0.006	—	0.012	0.080	—	—	—
10	0.008	—	0.010	0.031	—	—	—
11	0.006	—	0.024	0.063	—	—	—
12	0.007	—	0.020	0.066	—	—	—
13	0.008	—	0.006	0.095	—	—	—
14	0.003	—	0.008	—	—	—	0.020

Table 7-5. CONTENT OF TOTAL NITROGEN AND NITRATE-NITROGEN IN LEAVES OF FODDER SUGAR BEETS, EXPRESSED AS PERCENTAGE OF DRY WEIGHT¹¹

Sample	Total and nitrate-nitrogen, % of dry wt				Weighted mean
	Leaf position				
	Center	Inner	Middle	Outer	
Petioles					
Total N	3.80	2.03	1.73	1.89	1.87
NO ₃ -N ^a	0.23	0.45	0.74	1.28	0.83
NO ₃ -N, % of total	6.0	22.1	42.5	67.5	44.2
Blades					
Total N	5.70	4.80	4.15	3.60	4.10
NO ₃ -N	0.07	0.06	0.10	0.18	0.12
NO ₃ -N, % of total	1.2	1.4	2.4	5.0	3.0

^a NO₃-N is nitrate-nitrogen.

Table 7-6. DISTRIBUTION OF NITRATE-NITROGEN IN 2-INCH SEGMENTS OF LEAVES ON A SHOOT OF *PENNISETUM PURPUREUM*¹¹

Segment, inches	Approximate nitrate-nitrogen, ppm ^a		
	Outside (lowest) leaf	Fourth leaf	Eighth leaf
Sheath			
0-2	0	≥ 6	concealed
2-4	0	≥ 6	concealed
4-6	0	2	concealed
Blade			
0-2	0	1	1
2-4	—	0	≥ 6
4-6	—	0	≥ 6
6-8	—	0	2
8-10	—	0	2
10-12	—	0	0
12-14	—	—	0
14-16	—	—	0

^a Rough quantitative analysis with dimethylamine.

Nitrate is not uniformly distributed throughout plant tissues — stems usually contain more nitrates than leaves, and leaves more nitrate than flower parts. Roots have not been extensively tested but appear to contain lower levels than stems. In fodder sugar beets, the oldest leaves were found to be highest in nitrate nitrogen.

The site of nitrate reduction in plants is not definitely known. It has been suggested that in woody plants nitrate is reduced in the roots; in herbaceous plants, in the leaves.²⁷ McKee,²⁸ however, thinks that sufficient information does not exist for determining the site of reduction with any degree of certainty.

The activity of the enzyme, nitrate reductase, has been said to influence nitrate levels in plants. Energy is required for nitrate reduction, with carbohydrate the energy source. High levels of nitrogen appear to stimulate plants to utilize stored carbohydrate for energy with which to reduce the nitrate via nitrate reductase. As a result of rapid nitrate assimilation and rapid carbohydrate utiliza-

tion, the level of plant nitrate increases and the carbohydrate level decreases, so that a plant high in carbohydrate is likely to be low in nitrate.²⁷

Nitrogen uptake by plants is also influenced by the nitrogen content of the soil and by soil moisture, with potassium nitrate appearing to be more rapidly taken up than either calcium or sodium nitrate.²⁸

Molybdenum and manganese deficiencies have been shown to be associated with accumulations of nitrate in both plants and microorganisms. Molybdenum is the metallic component of the nitrate reductase enzyme.

Moisture-dependent processes are involved in the transformation and translocation of nitrate. Microbial activity, which releases nitrate from complex organic compounds, requires moisture. The nitrate released in this process also requires moisture to move through the soil to the plant roots and move across the cell membranes. Fertilizer nitrogen also requires moisture if a plant is to utilize it. Thus, plants that have been under a moisture-shortage stress may accumulate high levels of nitrate in a very few days. The moisture shortage results in a disturbance of assimilatory processes. As a result, a drop in nitrate reductase activity may occur while the plant continues to assimilate nitrates. Attempts to duplicate nitrate accumulation through experimental moisture stress have been few and not very successful.²⁷

A variety of other factors, such as light, herbicides, temperature, soil type, and parasitization by diseases and insects, have all been said to influence nitrate concentrations in plants.

Studies exist which indicate that spinach and lettuce can assimilate nitrosamines when they are present in the soil.¹⁶

7.1.5 Nitrosamines in Air

Theoretically, nitrosamines can be formed in the ambient air when secondary amines, nitrogen oxides, and sulfur dioxides are present in the air.²⁹

Sufficient data are not now available to determine the extent to which nitrosamines are formed in polluted or unpolluted atmospheres.

7.2 OCCURRENCE IN WASTE WATER³⁰

Since N-nitroso compounds are used as intermediates in certain industrial processes, N-nitrosamines may be found in waste water and sewage. An investigation in Baltimore, Maryland, and in Belle, West Virginia, has, in fact, shown the presence of nitrosamines in waste water.³⁰

Waste water samples taken from DuPont in the West Virginia study showed the presence of DMN at levels of 4.2, 2.3, and 2.4 $\mu\text{g/liter}$. A waste water sample from Fike Chemical Company in Nitro, West Virginia, also contained DMN at the 3.0 $\mu\text{g/liter}$ level. It is suspected that DMN levels in waste water arise from either DMN impurities in the amine process or subsequent nitrosation of the amine in the waste treatment facility.

A water sample taken from a sewage treatment facility adjacent to the Food Machinery and Chemical Corporation (FMC) plant in Baltimore contained 2.7 $\mu\text{g/liter}$ of DMN. The plant produces unsymmetrical dimethylhydrazine (UDMH). On the FMC property, close to the UDMH plant, a rain-water puddle contained over 6000 $\mu\text{g/liter}$ of DMN. A mud sample in the same area contained over 200 $\mu\text{g/liter}$. A water sample from a drainage ditch on the border of the FMC property with an adjacent firm (British Petroleum) contained 5.9 $\mu\text{g/liter}$. Water taken directly from an adjacent cove maintained DMN levels in the 0.26 to 0.94 $\mu\text{g/liter}$ range.

7.3 INDUSTRIAL SOURCES OF OCCURRENCE

7.3.1 Industrial Processes in Which Nitrosamines Occur as Primary Product or Intermediate

Although a large patent literature exists on nitrosamines, these compounds do not appear to have been used widely. Patent applications reveal potential use in the manufacture of rubber, dyestuffs, gasoline additives, lubricating oils, explosives, insecticides, fungicides, dielectric fluids, acrylonitrile, plasticizers, industrial solvents and hydrazine.³¹ Apart from the variety of potential applications described in the patent literature, three major industries are involved in the handling of nitrosamines today, organic chemicals manufacturing, rubber processing, and rocket fuel manufacturing.

A review of *Chem Sources-U.S.A.*³² reveals that a large variety of nitrosamines (including dimethyl-, diethyl-, dipropyl-, dibutyl-, and dipentyl nitrosamine) are available commercially. The only nitrosamines produced in quantities greater than 1000 pounds per year are diphenylnitrosamine and dimethylnitrosamine.

Thus far, diphenylnitrosamine has not been shown to be carcinogenic, and consequently is of little interest here. The rubber industry requires chemicals to modify the basic elastomers to produce the desired improved qualities in rubber goods. Such chemicals, including monomers, amount to more than 5.5 billion pounds per year.³³ Of the variety of different types of chemicals used, two types are pertinent to this discussion, vulcanizing retarders and blowing agents. Diphenylnitrosamine (not carcinogenic) is a well-known vulcanizing retarder and dinitrosopentamethylenetetramine is used as a blowing agent in the production of microcellular rubber.³¹

One of the main liquid rocket fuels has been 1,1-dimethylhydrazine. A high-yield procedure for preparing unsymmetrical hydrazines involves the reduction of the corresponding nitrosamine precursor. In the commercial process, 1,1-dimethylhydrazine is prepared in a 73-percent yield by reducing DMN with zinc and acetic acid.³⁴



The FMC plant in Baltimore is a commercial source that produces DMN as an intermediate in the production of 1,1-dimethylhydrazine.³⁵

7.3.2 Industrial Sources in Which Nitrosamines Occur Incidentally

Dimethylnitrosamine and N-4-dinitrosomethylaniline have been suspected to be related to the higher-than-normal mortality from cancer in rubber factory workers.³⁶ However, the use of these two compounds *per se* in rubber processing is doubtful; it is more likely they originated from the corresponding amines, which may have been used as antioxidants.

Lower-molecular-weight nitrosamines are volatile at room temperature while higher-molecular-weight species are steam volatile. Because of possible loss of these compounds from complex reaction mixtures by volatility and also because of the diversity of the nitrogen-based organic chemical industry, it is reasonable to expect that many more sources of nitrosamines to the atmosphere will be identified.

Nitrosamines have been demonstrated to be present in the following products and processes, and, as a result, may contribute to ambient air contamination during their manufacture or use. Listed also are examples of industries where the occurrence of nitrosamines has not been documented but could be expected.

- Combustion of rocket fuel. There is some evidence that nitrosamines are formed during the burning of 1,1-dimethylhydrazine as a rocket fuel.³⁷
- Fish meal processing. The presence of 30 to 100 ppm of DMN has been found in fish meal, which also contains relatively large amounts of secondary and tertiary amines.³⁸

- Soya bean oil. DMN has been found also to the extent of 0.38 to 0.45 ppm in one source of soya bean oil.^{39,40}
- Tobacco. Nitrosation of the secondary amine nornicotine (its ratio to nicotine in tobacco is about 1:20) may contribute to the occurrence of NO-nornicotine in tobacco smoke and in cured, unsmoked tobacco.^{41,42} There are some data to suggest that smoke from tobacco grown in soil treated with high levels of nitrogen is more likely to contain nitrosamines and to contain them in higher concentrations than smoke from tobacco grown in the fields with a low nitrogen content. Evidence for the presence of DMN, nitrosopyrrolidine, methylbutyl-nitrosamine and nitrosopiperidine in tobacco smoke has been obtained.⁴³⁻⁴⁶
- Explosives manufacture. High explosives contain various organic nitrates as the main component of the explosive. However, a multitude of various additives is also used to impart specific properties to the explosives. Pertinent to the discussion here are stabilizers (secondary amines, e.g., diphenylamine); binders (natural or synthetic nitrogenous materials); compatibility agents and softening point depressants (nitrosophenols and their esters); and fuel sensitizers for ammonium nitrate (amines, amides, urea).⁴⁷
- Power plants. It has been suggested that amine additives used to modify fly-ash resistivity might possibly react with flue gas NO_x to form nitrosamines. An unpublished report,⁴⁸ however, cites evidence to the contrary. No nitrosamine was detected in tests at a power plant at which diethylamine sulfate was used as the additive (the detection limit was calculated to be 5 parts per trillion). The sampling method employed a TENAX adsorber, and sampling volume was approximately 50 cubic feet. Organics were desorbed from TENAX using pentane, and aliquots were analyzed by gas chromatography-mass spectrometry after concentration by Kuderna-Danish evaporation.

7.3.3 Industrial Sources of Nitrosamine Precursors

7.3.3.1 NO_x Sources—An estimated 20.69×10^9 kg of nitrogen oxides were emitted from industrial, commercial, and domestic sources in the United States during 1970. Table 7-7 shows the contribution to this total from each source category.⁴⁹

About 1 percent of the total man-made NO_x emitted to the ambient air is formed by chemical processes, mainly related to the manufacture and use of nitric acid. Use of nitric acid in military ordnance works for explosives manufacture is a major source of NO_x emissions, with total NO_x emissions from the Volunteer Ordnance Works equal to total emissions from all U.S. non-military uses of nitric acid.⁴⁹

Nitrogen oxides emissions from organic nitrations are shown in Table 7-7 under industrial processes.

7.3.3.2 Secondary Amine Sources—A detailed review of all the specific unit operations and processes involving the use or generation of secondary amines is beyond the scope of this report. The two sources described below may be expected to emit secondary amines.

- Feedlots. Dimethylamine has been identified in the air from the decomposition of livestock and poultry manure.⁵⁰ At least eight amines (methylamine, dimethylamine, trimethylamine, ethylamine, triethylamine, *n*-propylamine, *n*-butylamine, and *n*-hexylamine) have been identified in the air from decomposition of livestock and poultry manure. A survey⁵¹ of two Texas panhandle feedlots indicated the presence of the following amines in a 20.6-liter sample:

Compound	Wt collected, μg
Ethylamine	2.7
Dimethylamine	13.0
<i>n</i> -Propylamine	1.5
<i>n</i> -Butylamine	1.2
<i>n</i> -Hexylamine	0.7

The concentration of dimethylamine in air above these feedlots (at 18 m) is calculated to be $632 \mu\text{g}/\text{m}^3$.

**Table 7-7. SUMMARY OF NITROGEN OXIDES
EMISSIONS IN THE UNITED STATES, 1970⁴⁹**

Source	10 ⁹ kg/yr	Percent of total
Transportation	10.6	51.3
Motor vehicles	8.3	39.9
Gasoline	7.1	34.2
Diesel	1.2	5.7
Aircraft	0.36	1.8
Railroads	0.09	0.4
Vessels	0.18	0.9
Non-highway use of motor fuels	1.7	8.3
Fuel combustion in stationary sources	9.1	43.8
Coal	3.5	17.1
Fuel oil	1.2	5.7
Natural gas	4.1	19.7
Liquid petroleum gas and kerosene	0.18	0.9
Wood	0.09	0.4
Industrial processes	0.18	0.9
Solid waste disposal	0.36	1.8
Miscellaneous	0.45	2.2
Forest fires	0.18	0.9
Structural fires	Negligible	Negligible
Coal refuse	Negligible	Negligible
Agricultural	0.27	1.3
Total	20.69	100.0

The secondary amines found in feedlots are of primary interest in the formation of nitrosamines. Tertiary amines can degrade to secondary amines; however, this adds another reaction step and slows the kinetics of nitrosamine formation from tertiary amines. There are two routes possible to nitrosamine formation in feedlots, (1) reaction of amines with nitrite in wastes and subsequent emission by volatilization or on dust; and (2) atmospheric reaction of feedlot-generated amines with NO_x . Certainly more work needs to be done to establish feedlots as a possible source of nitrosamines. The recent explosive growth of feedlots in some areas of the U.S. of up to 18 percent per year reinforces the need for investigation.

Pertinent to the problem of wastes in feedlots, nitrosamines were found after incubating dimethylamine and nitrite with microorganisms derived from sewage and soil with whole acidic soil and sewage.⁵² Under these conditions, trimethylamine was also converted to dimethylamine. Amines and high concentrations of nitrites are commonly found in soils and sewage.^{50,53}

- Rendering plants. Animal matter not suitable as food for either humans or pets is rendered into salable products. Ground animal matter is conveyed to cookers which may handle as much as 40,000 pounds in batch processes. Temperatures of 300°F for 1 to 4 hours are required to digest bones, hooves, hides, and hair, with most of the moisture being evaporated and exhausted. This exhaust steam contains extremely odorous gases. Amines that have been identified in the exhaust gases are: trimethylamine, ethylamine, diethylamine, triethylamine, putrescine ($\text{H}_2\text{N}(\text{CH}_2)_4\text{NH}_2$), and cadaverine ($\text{H}_2\text{N}(\text{CH}_2)_5\text{NH}_2$).⁵⁴ It has not been established whether exhaust gases contain nitrosamines. Since the tissues of animals contain dimethylamine and trimethylamine almost ubiquitously as metabolic or bacterial degradation products of nitrogen metabolism, and since nitrosamines have been shown to form from amines and nitrite in the alimentary tract of animals, the presence of nitrosamines in the offal of animals is a possibility. At the high cooking temperatures, many nitrosamines would be volatilized and practically all nitrosamines are steam-distillable.⁵⁵

The manufacture of the following products or the products themselves may be sources of nitrosatable secondary amines.

- Antioxidants. Dimethylamine is used in liquid tetrafluoroethylene. Ethylamine is injected in the air in which rubber products are stored to inhibit degradative oxidation.⁵⁶ Various aryl and alkyl amines are used to inhibit the oxidation of lubricants.⁵⁷
- Vulcanization accelerators. Large quantities of amines are used in the preparation of rubber vulcanization accelerators. Secondary amines are used in the preparation of thiuram sulfides ($R_2N-CS-SS-CS-NR_2$) and metallic dialkyldithiocarbamates ($R_2NCSSNa$).⁵⁸
- Pharmaceuticals. The applications of aliphatic amines in the preparation of pharmaceuticals are too numerous to list. Some examples are: (1) ephedrine [$CH(OH)CH(CH_3)NHCH_3$], an adrenergic drug used as a bronchodilator; (2) diethylamine, used in the preparation of chloroquine; (3) di-*n*-propylamine, used in the preparation of Benemide, an adjunct to penicillin therapy; and (4) amines used in germicidal preparation.⁵⁸
- Self-polishing waxes. Morpholine, together with fatty acids, is used as an emulsifying agent for self-polishing waxes.⁵⁸ Morpholine evaporates as the film dries.
- Synthetic detergents. Dimethylamine is a contaminant in detergents.⁵⁹
- Pesticides. Secondary amines are used extensively in the preparation of herbicides, fungicides,⁵⁸ and insecticides. Many pesticides contain secondary and tertiary amine groups and might be expected to form nitroso derivatives. Discussion has been given earlier in this report concerning nitrosation of pesticides (see Sections 3.2.3, 4.4, 5.2, 5.3.3).

Pesticides may be discussed in terms of a number of generic groups. The groups which are most interesting from the standpoint of containing possible nitrosamine precursors include: (1) triazines (e.g., atrazine); (2) carbamates (e.g., Sevin); (3) thiocarbamates (e.g., Addicarb); (4) hydrazides (e.g., maleic hydrazide); (5) urea-based compounds (e.g., Bromicid); (6) nitrated hydrocarbons (e.g., Trifluralin); and (7) amides (e.g., Diphenamid).
- Solvents. An important solvent for acrylonitrile polymers and copolymers, dimethylformamide, is made from methylformate and dimethylamine. About 14 million pounds of dimethylamine are used annually.⁵⁸
- Uranium oxide. Amines such as dilaurylamine are used in the solvent extraction of uranium oxide from ore-leach-liquors.⁶⁰
- Wet-strength paper. Polyethylenimine is used in paper to improve its wet strength.⁵⁸
- Corrosion inhibitors. Morpholine is used as a corrosion inhibitor.⁵⁸
- Animal glues. Amines are emitted during cooking and drying operations.⁶¹
- Photographic products. Amines are used as developing agents, e.g., *p*-methylaminophenol.³³
- Leather. Dimethylamine is used as an accelerator in the liming operation involved in the tanning of leather.³³

The above list of amines which may be precursors to nitrosamine formation is by no means complete. The number of permutations on a fundamental organic structure is immense, as illustrated by the group of related compounds shown in Figure 7-4. It should also be mentioned that chlorophyll and heme contain the basic pyrrole structure.

The discussion of pyrrole-type compounds was given to point out that no exhaustive list of possible amine precursors to nitrosamines can be given in this report. The report can, at best, cite examples of possible precursors and note those compounds important in commerce that should be examined to determine whether they could be important sources of nitrosamines to the environment.

Table 7-8 gives a list of amine compounds that have been found to yield nitroso derivatives.⁶² A partial list of United States amine producers is given in Table 7-9.⁶² Given in Table 7-10 is a selected list of some of the larger-volume amines which are produced along with production figures.⁶² The list of production figures is not complete and consequently is of limited value, but it does offer some

**Table 7-8. DRUGS, PESTICIDES, AND NATURALLY
OCCURRING AMINES THAT HAVE EXPERIMENTALLY
PRODUCED N-NITROSO COMPOUNDS *IN VIVO*
OR *IN VITRO*^{a,62}**

Compound	Class ^b	Product
Secondary amines		
Morpholine	D,P	NO-derivative
Piperazine	D	MNP,DNP
Atrazine	P	NO-derivative
Simazine	P	NO-derivative
Phenmethazine	D	NO-derivative
Ethambutol	D	NO-derivative
Ziram	P	DMN
Thiram	P	DMN
Ferbam	P	DMN
Tertiary amines		
Aminopyrine	D	DMN
Oxytetracycline	D	DMN
Chlorpromazine	D	DMN
Dextropropoxyphene	D	DMN
Chlorpheniramine	D	DMN
Methadone	D	DMN
Methapyrilene	D	DMN
Quinacrine	D	DEN
Lucanthone	D	DEN
Tolazamide	D	NO-hexamethyleneimine
Cyclizine	D	DNP
Trimethylamine	N	DMN
2-Dimethylaminoethanol	N	DMN
N,N-Dimethylglycine methyl ester	—	DMN
N,N-Dimethylglycine	N	DMN,NO-sarcosine
Triethanolamine	—	NO-diethanolamine
Nitrilotriacetic acid	—	NO-iminodiacetic acid
N,N-Dimethyldodecylamine	—	DMN,NO-N-methyldodecylamine
Nicotine	N	NO-nornicotine
Pyribenzamine	D	NO-derivative
Carbaryl	P	NO-carbaryl
Propoxur	P	NO-propoxur
Benzthiazuron	P	NO-benzthiazuron
Quaternary amines		
Tetramethylammonium chloride	—	DMN
Neurine chloride	N	DMN
Acetylcholine	N	DMN
Choline	N	DMN
Betaine	N	DMN
Carnitine	N	DMN
Trimethylamine-N-oxide	N	DMN
Tribenzylamine-N-oxide	—	Dibenzylnitrosamine

^a Adapted from S. S. Mirvish. Formation of N-nitroso compounds: chemistry, kinetics and *in vivo* occurrence. *Tox. and Appl. Pharmacol.* 31, 1975.

^b D = drug; P = pesticide; and N = naturally occurring.

^c NO-derivative of the compound nitrosated.

Table 7-9. PRODUCERS OF AMINES IN THE UNITED STATES⁶²

ACCTO CHEMICAL COMPANY Charlestadt, New Jersey	MILES LABORATORY Elkhart, Indiana
AIR PRODUCTS & CHEMICALS, INC. Pensacola, Florida	MILLMASTER ONYX CORPORATION Beaumont, Texas
ALDRICH CHEMICAL COMPANY, INC. Milwaukee, Wisconsin	MONSANTO Luling, Louisiana
AMES LABS Milford, Connecticut	NALCO CHEMICAL COMPANY Chicago, Illinois
ARMAK COMPANY Chicago, Illinois	NEASE CHEMICAL CORPORATION State College, Pennsylvania
BASF WYANDOTTE CORPORATION Parsippany, New Jersey	PENNWALT CORPORATION Wyandotte, Michigan
CELANESE CORPORATION Bay City, Texas	PIERCE CHEMICALS, INC. Rockford, Illinois
COMMERCIAL SOLVENTS CORPORATION Terra Haute, Indiana	R.S.A. CORPORATION Ardsley, New York
DOW CHEMICAL COMPANY Freeport, Texas	REILLEY TAR & CHEMICAL CORPORATION Indianapolis, Indiana
EASTMAN KODAK COMPANY Kingsport, Tennessee Rochester, New York	ROHM & HAAS Philadelphia, Pennsylvania Deerpark, Texas
E. I. DuPONT de NEMOURS, INC. Belle, West Virginia Houston, Texas	SHELL CHEMICAL COMPANY Martinez, California
EL PASO PRODUCTS Odessa, Texas	STAUFFER CHEMICAL COMPANY Edison, New Jersey
ELI LILLY & COMPANY, INC. Lafayette, Indiana	STERLING DRUG COMPANY Cincinnati, Ohio
GAF CORPORATION Calvert City, Kentucky Linden, New Jersey Rensselaer, New Jersey	UNION CARBIDE CORPORATION Institute, West Virginia South Charleston, West Virginia Taft, Louisiana Texas City, Texas
HILTON-DAVIS CHEMICAL COMPANY Cincinnati, Ohio	UNIROYAL Naugatuck, Connecticut
JEFFERSON CHEMICAL COMPANY Austin, Texas Conroe, Texas Port Neches, Texas	VIRGINIA CHEMICALS Portsmouth, Virginia
	WARNER LAMBERT Holland, Michigan

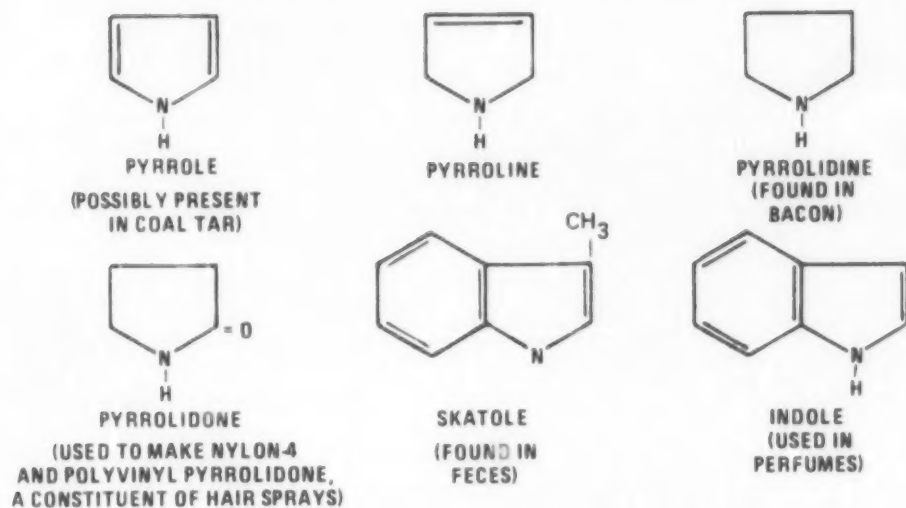


Figure 7-4. Structures and diverse sources or uses of pyrrole and related compounds.

Table 7-10. AMINE PRODUCTION IN THE UNITED STATES, 1972⁶²

Amine	Production, tons (10 ⁶)
Diethanolamine	4.7
Hexamethylenamine	30.65
N-(1,4-dimethylpentyl)-N'-phenyl-p-phenylenediamine	1.0
Aniline	19.9
Triethanolamine	4.15
Ethanolamine	11.0
Dimethylamine	4.75
Diarylatediamines, mixed	1.0
Hexamethylenetetramine	4.75
Melamine	3.4
Ethylene diamine	3.1
Toluene, 2,4-diamine	6.65
Monomethylamine	1.65
Trimethylamine	1.4
Dipropylamine	1.35
Di-n-butylamine	0.19
Diethylamine	0.55
All other ethylamines	2.2
Total butylamines	0.95
Atrazine	4.5

perspective. It is impossible, with the present data base, to develop an understanding of the importance of amine manufacturing as a source of nitrosamines precursors compared to other sources in which amines are incidental to the existence and purpose of the source.

All of the companies listed in Table 7-9 were contacted in an effort to procure data from each indicating quantity of production. At this time, only three of the responding companies have made production figures available, as shown in Table 7-11. Using the 1975 *Directory of Chemical Producers*, the production capacities for several companies were procured (also given in Table 7-11).

**Table 7-11. ACTUAL PRODUCTION OR PRODUCTION CAPACITY
OF SOME AMINE PRODUCERS IN THE UNITED STATES**

Producer	Compound	Actual production or production capacity
<i>Actual production:</i>		
Aldrich Chemical Company, Inc.	<i>dl</i> - α -Methylbenzylamine	90 to 175 kg/yr each of last 5 yr
Ames Laboratories	<i>p</i> -Methylbenzylamine	20 to 50 lb/yr each of last 5 yr
Virginia Chemicals, Inc.	Dipropylamine	11,100,000 lb
	Tripropylamine	42,000 lb
	Dibutylamine	1,523,000 lb
	Tributylamine	150,000 lb (1974 figures)
<i>Production capacity:</i>		
GAF Corporation	Mono-, di-, and trimethylamine	10,000 lb
E. I. DuPont de Nemours, Inc.	Mono-, di-, and trimethylamine	165,000,000 lb
Commercial Solvents Corporation	Mono-, di-, and trimethylamine	18,000,000 lb
Air Products & Chemicals, Inc.	Mono-, di-, and trimethylamine	50,000,000 lb

7.4 MOBILE SOURCES

Studies have been conducted on nitrosamines in exhaust. The exhaust from three 1972 model cars and two engines operated on dynamometer stands was analyzed for a variety of nitrogen-containing compounds, including dimethylnitrosamine, in a fuel-additive study.⁶³ A study of the impact on emissions of six fuel additives in five 1973 and 1974 model cars involved analysis for amines and nitrosamines.⁶⁴ A method of analysis (N-specific gas chromatography) for amines and nitrosamines was developed as a part of a program on fuel additive effects,⁶⁵ and this technique was used in fuel

additive product analysis in flat-flame burner experiments.^{66,67} A study of the composition of particulate matter emitted from diesel engines involved analysis for absorbed amines and nitrosamines using a variety of fuels and additives.⁶⁸ Another fuel additive program used gas chromatographic and mass spectrometric techniques to search for N-nitrosamines from engines and combustion bombs, using simple fuels and high levels of simple nitrogen-containing additives.⁶⁹ Sensitive spot tests for nitrosamines were used in a study of the particulate emissions from four monolithic oxidation catalysts, three pelleted oxidation catalysts, and two NO_x reduction catalysts.⁷⁰

Thus far, analyses for nitrosamines and amines have been carried out as major parts of seven research programs involving many automobiles, diesel engines, emission control systems, fuels, and fuel additives. No evidence for either amine or nitrosamine emissions has been developed from any tested sources in any of the studies found to date.

Analysis for nitrosamines in low concentrations in source emissions is a task of high complexity. N-nitroso compounds are relatively unstable species at source effluent temperatures, and there are many compounds of potential concern. In most cases, surveys of source emissions have searched only for N-nitrosodimethylamine, the lowest member of the homologous series of N-nitrosated secondary amines. Most workers have used nitrogen-specific detectors in conjunction with gas chromatography to determine this compound.

Since emitted amines can be N-nitrosated in the ambient air, analysis for the potentially complex variety of potential amine emission products is also important. Again, the most commonly used tool has been gas chromatography with N-specific effluent detection. This class of compounds has been one of the most difficult of all organic species to handle. Extra precautions, including all-glass gas handling systems, super-inert chromatographic substrates, and relatively high-temperature separations, have all been necessary to avoid serious losses of model compounds in standardization experiments. In one study, new methods of detection, handling, and analysis have been extensively explored and the experimenters' efforts have been met with moderate success. However, analysis for a variety of amines and nitrosamines from model combustion systems has been entirely negative.

It should be recognized that the initial publication of the N-specific detector system used in the recent work of Fine and Epstein on nitrosamines in ambient air resulted from work done under an EPA grant.⁶⁵

Further discussion of source emission studies follows.

7.4.1 Bureau of Mines Studies

The gaseous emissions from three 1972 Chevrolet automobiles and two stationary 1972 Chevrolet engines were analyzed for nitrogen compounds using both clear gasoline and gasoline containing Chevrolet additive F-310, an amine-type carburetor detergent. Compounds analyzed included ammonia, pyridine, C₂ to C₄ aliphatics, N-nitrosamines, aniline and toluidine N-nitrosamines, cyanogen, hydrogen cyanide, and nitromethane. Detection methods used included electron capture, alkali flame-ionization, microcoulometry, and electrolytic conductivity. This last method had the best overall selectivity and sensitivity and was used for the major analytical effort. Detection limits for auto exhaust for compounds of interest ranged from 20 ppb for aromatic amines to 150 ppb for nitrosamines. Of the compounds searched for, only HCN (1.0 to 1.5 ppm) and nitromethane (0.2 to 0.3 ppm) were confirmed as present in automobile exhaust. All others were absent or were present at levels below detection limits.

In later work at the Bureau, six additives, including F-310 in normal and high aromatic base fuels, were examined in 1973-1974 model cars (a Ford, a Chevelle, a Volkswagen, and two Mazda rotary engine cars), using the same techniques. Detection limits were extended to about 25 ppb for all compounds of interest. Compounds found to be present in ppb quantities included HCN, cyanogen, and alkyl and aryl nitro compounds. Further, experiments were conducted in which automobile exhaust was spiked with known concentrations of ammonia, alkyl and aryl amines, nitrites, and nitrosamines. Auto exhaust samples in light-proof bags were spiked with the compounds of interest to several times the detection limit. After 30 minutes, less than 30 percent of the original amounts remained. The results of all these vehicle experiments showed that all this latter class of compounds

are unstable and undetectable in auto exhaust. None of the nitrogen compounds found were elevated with use of N-containing additives.

7.4.2 Southwest Research Institute (SWRI) Studies^{67,68}

Experiments at Southwest Research Institute sought nitrogen compounds from catalyst-equipped engines. Using techniques similar to those developed by the Bureau of Mines, nitrogen compounds were sought in the exhaust of non-air-pumped pelleted catalysts with two base gasolines and two N-containing additive packages. No nitrogen compounds were found at 25 ppb detection limit.

Further experiments were conducted at SWRI in an effort to identify N compounds possibly adsorbed on diesel particulate emissions. Tested were both two-stroke and four-stroke cycle diesel engines, using three widely different diesel base fuels and two additives, one of them an organic nitrite ester used as an ignition promoter. Again, no nitrogen-containing products were detected.

7.4.3 Exxon Studies⁷⁰

Particulate emissions from eight catalyst systems were characterized by Exxon Research. Particulate samples were examined by spot-plate tests for nitrosamines and amines.

7.4.4 University of Michigan Study^{65,66}

Under a study at the University of Michigan, a super-sensitive, N-sensitive detection system involving chemiluminescence detection has been developed and reported.^{65,66} Chromatographic techniques for the analysis of a wide variety of amines and nitrosamines were also developed and carefully standardized. Studies of partial combustion products from amine antioxidants were performed on a simplified combustor, but no N-containing compounds were found.

7.4.5 Penn State Study⁶⁹

A gas chromatograph-mass spectrometry study of the composition of auto exhaust with and without very high levels of amine antioxidants was carried out at the Pennsylvania State University. No N-containing products were identified.

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8. CONTROL TECHNOLOGY

8.1 CONTROL TECHNOLOGY FOR STATIONARY SOURCES OF NITROSAMINES

8.1.1 Introduction

Nitrosamines as a class of compounds can exist in the gaseous, liquid, or solid state, depending on molecular weight. They can be emitted to the environment in any of the phases or as a solute in water or hydrocarbons. They can be adsorbed in solid particles or absorbed in aerosols, and can also be formed in the environment by the reaction of secondary or tertiary amines with nitrous acid or nitric oxides.^{1,2}

Because of the wide range of physical properties and sources of nitrosamines, control technology requirements for many types of sources are discussed. Data are limited since nitrosamines are not widely manufactured. The control techniques discussed are therefore based on the application of general principles commonly used for the control of many types of pollutants. Limited experimental data on control of nitrosamines in water are presented in detail. Because of the possibility of *in situ* nitrosamine formation in air, control of the precursors is covered briefly.

8.1.2 Control Technology for Air Emissions of Nitrosamines

Most nitrosamines are liquids having relatively high boiling points and, as such, are not found in large quantities in the gaseous state. However, small amounts at the ppb or ppm level can exist in equilibrium with the liquid. Because of the low vapor levels possible and the limited use of nitrosamines in the chemical industry, very little has been done to explore vapor-phase control techniques. Methods suggested are based on the known properties of the materials and on engineering judgment.

8.1.2.1 Wet Scrubbing—The two lowest-molecular-weight nitrosamines (dimethyl and diethyl) are soluble in water, though other compounds in this class are insoluble. Consequently, water scrubbing can be used as an effective control technique in the case of dimethyl- and diethylnitrosamine. For higher-molecular-weight nitrosamines, wet scrubbing is not an efficient control method except when the aqueous medium contains a soluble oxidizing agent such as hydrogen peroxide, potassium permanganate, or chlorine.^{3,4} The efficiency of removal would depend on the rate of oxidation, which is a function of the oxidizing agent and the pH of the scrubbing medium. The oxidation rate is also affected by temperature and pressure, the presence of catalytic species, and the efficiency of utilization of oxidant. Catalytic species can increase the oxidation rate or cause premature decomposition of the oxidizing agent, resulting in poor utilization of the oxidizing agent. Discussion given in Section 8.1.3 indicates that under drastic conditions of temperature and pressure wet-air oxidation is more effective than oxidation with hydrogen peroxide, a stronger oxidant. Unfortunately, there are practical limitations to the temperature and pressure conditions that may be employed in a continuous scrubbing process. The presence of other oxidizable species in the effluent gas stream could result in competitive reactions that could reduce the efficiency of the system and increase the cost of control. It is possible that scrubbing reaction products may in themselves present environmental problems. For example, the use of chlorine as an oxidant could result in the formation of organochloramines or chlorinated hydrocarbons. Further work is necessary to determine the most suitable oxidant.

The cost of control technology is to a great extent a function of the reaction rate (which affects the size of equipment) and the required control efficiency. These parameters have not been defined.

Typical capital costs for scrubbers used for particulate removal run from \$0.25 to \$3.00 per standard cubic foot per minute (scfm) capacity.

8.1.2.2 Incineration—Control of nitrosamine emissions by high-temperature oxidation would be an efficient method. Selection of its use would depend on nitrosamine concentration, the moisture content of the effluent gas stream, the constancy of emission, and the cost and availability of fuel.

Capital costs⁵ for conventional incinerators without heat exchangers may be expected to be \$2.00 to \$3.00 per scfm. Heat exchangers increase capital costs by a factor of two. Operating costs may vary from \$3.00 to \$30.00 per hour over the capacity range of 5,000 to 38,000 scfm.

It may be possible to reduce the operating temperature, and thus the costs of fuel, for catalytic incineration. Work is being done to develop better catalysts, and their successful development could make this a practical method of control at some future date. Capital costs for catalytic units may be expected to be about 1/10 those for conventional incinerators. However, operating costs for catalytic units may be less than half those for conventional units.

8.1.2.3 Adsorption—Activated charcoal may be used to adsorb nitrosamines from effluent gas streams. In instances where the moisture content of the effluent gas is high, the temperature of the gas is also usually high and a condenser must then be used to reduce the gas temperature (and moisture content) since adsorption is inefficient at high temperatures ($>120^{\circ}\text{F}$).⁶ In these instances, the bulk of the nitrosamines would be condensed with the water and the adsorber would take out the residual. It has been estimated⁷ that adsorption preceded by condensation is as efficient a control method as the use of an afterburner (incinerator) preceded by a condenser. Capital costs for carbon sorption may be expected to be \$2.00 to \$8.00 per scfm capacity.

8.1.2.4 Catalytic Reduction—Reduction of nitrosamines to hydrazines is a well-documented reaction. The development of catalysts to improve the efficiency of this reduction could make this a practical control method. Investigations on the use of a copper-iron couple as the reducing agent may lead to useful information. This reduction system has been successfully applied to chlorinated pesticides and polychlorinated biphenyl compounds in manufacturing waste waters. Possibly this system could be used in combination with a water scrubber to reduce the nitrosamines captured in the scrubbers to hydrazines. Capital costs for waste water treatment are projected to be \$1,000 per gallon per minute capacity; operating costs are projected to be negligible.

8.1.2.5 Ultraviolet Radiation—Nitrosamines are known to degrade photochemically both in air and in aqueous solution.² It may be possible to take advantage of this reaction to control the emission of nitrosamines. The degradation products would be expected to be the parent compounds (e.g., amines and nitrite). The quantum yield for this reaction is unlikely to be high enough to allow ultraviolet radiation to be used as an effective control technology for atmospheric emissions. The practical consideration of retaining large volumes of waste gases for the required long irradiation times would make ultraviolet irradiation a difficult approach. However, if the quantum yield were high enough, this method could be the simplest and most economical control technique for removing nitrosamines from effluent gases.

8.1.3 Control Technology for Nitrosamines in Industrial Waste Water

Data concerning the control of nitrosamines in water are relatively scarce. Many laboratory investigations relative to the chemical properties of nitrosamines hold out several possible decontamination techniques, but to date none has proved to be effective in practice. In fact, only in the case of UDMH rocket-fuel production waste (U.S. Air Force) have actual treatment studies been conducted with the goal of complete elimination of a nitrosamine (dimethylnitrosamine). These studies, while not yet totally successful, point out that a combination of techniques will probably have to be utilized in order to achieve complete detoxification.

Several methods for destroying nitrosamines in water have been suggested. These include oxidation by chlorine, hydrogen peroxide, ozone, or wet air; reduction by sodium hydrosulfite; photochemical degradation; and biodegradation. In addition, suggested methods for separating or removing nitrosamines from water include: (1) steam stripping/distillation, followed by recycle and reuse of

the nitrosamine; and (2) carbon adsorption, followed by incineration or thermal regeneration of the carbon.

8.1.3.1 Oxidation by Chlorine—The data generated by the Food Machinery and Chemical Corporation, Baltimore, Maryland, for the U.S. Air Force on the caustic waste generated from the production of UDMH for rocket fuel have indicated that chlorination is capable of removing from 50 to 95 percent of the DMN (initial concentration, 12 percent), depending on the amount of sodium hypochlorite used. When a sevenfold excess of sodium hypochlorite was added to a 125-ppm DMN solution, 95 percent removal was achieved within 30 minutes.

Gas chromatographic-mass spectrometric analysis (GC-MS) revealed the presence of chloromethane (CH_3Cl) and dichloromethane (CH_2Cl_2) in chlorination products of DMN; and these compounds, along with chloroform, carbon tetrachloride, and chlorinated ethanes, in the chlorination products of UDMH. Based on the presence of these chlorinated hydrocarbons, which, like DMN, are suspected carcinogens, it does not appear that chlorination is a feasible treatment method.

8.1.3.2 Oxidation by Hydrogen Peroxide—The laboratory experiments by FMC Corporation for the U.S. Air Force have shown that in acidic solutions 40 percent of the DMN was removed by hydrogen peroxide after a 1-hour reflux at 100°C . Hydrogen peroxide oxidation, therefore, should not be considered an effective treatment method.

8.1.3.3 Oxidation by Ozone—Since hydrogen peroxide does not effectively remove DMN from aqueous solution, a stronger oxidizing agent must be employed. However, as can be seen in the case of chlorination (where the presence of chlorinated hydrocarbons after treatment precludes its use), the oxidizer must also react to form innocuous products and byproducts. Ozone can be expected to possess these critical properties, but no firm data exist on what reaction conditions are necessary to achieve essentially complete removal. Possible use of ozonation in conjunction with ultraviolet (UV) photolysis may also be beneficial.

Since commercial waste water treatment systems are available that use ozonation with or without UV photolysis, these options should be considered and investigated further in the control and removal of DMN from the water environment.

8.1.3.4 Oxidation by Wet Air—Laboratory data gathered by Zimpro, Inc., for the Air Force have shown that wet air oxidation (wet-ox) is successful in reducing the DMN concentration in waste water from 400 ppm to less than 1 ppm within one hour at 300°C at 3000 psi. Unfortunately, no pilot-scale or full-scale experiments have yet been conducted to assess the full potential of this promising technique. The residence time and high pressure requirements would make this approach very expensive.

8.1.3.5 Reduction by Sodium Hydrosulfite—The laboratory experiments by FMC Corporation for the U.S. Air Force have been conducted to determine the extent of the reduction of DMN to dimethylamine by sodium hydrosulfite. Results indicate that, in alkaline solution, 60 percent of the DMN (initial concentration, 1.5 percent) was removed after 2 hours at 50°C . This method, therefore, is not effective enough to warrant further attention.

8.1.3.6 Photodegradation—Data on the photochemical cleavage of the nitroso group from nitrosamines in aqueous solution indicate that for very low concentrations (25 ppm) virtually 100 percent of the nitrosamine is converted to the amine by irradiation at 230 nm (UV light); but, as the concentration of nitrosamine is increased, the extent of cleavage is decreased.⁸ It appears, therefore, that photodegradation will be effective only on wastes that have been pretreated by some other technique to reduce nitrosamines to a very low residual concentration.

8.1.3.7 Biodegradation—The very fact that DMN is formed as a metabolic product in many species and does not bioaccumulate leads one to suspect that it is susceptible to bacterial degradation. Few data are available, however, to indicate to what degree, how fast and under what conditions a biological treatment system can remove the material from the waste stream or what the byproducts of such removal would be. Because of this lack of data and the nature of the DMN, it is recommended that a waste stream containing this compound not be channeled to a municipal or joint municipal-

industrial sewage treatment plant unless pilot-scale testing of the waste shows that the DMN is totally removed.

8.1.3.8 Stream Stripping/Distillation—One method of separating nitrosamines from an aqueous waste stream is to distill the material and recycle it to the process. The FMC Corporation studies with DMN (for the U.S. Air Force) showed that this method could reduce the concentration from 1180 ppm to 60 ppm when 8 percent of the waste water was allowed to distill over with the DMN. Stream stripping of the solution was considered to be a pretreatment method. Actual use of the technique is under way at the FMC UDMH plant, where the DMN concentration of the waste is reduced from the 1.5 percent range to the 400 ppm range. However, 400 ppm is probably not an environmentally acceptable DMN concentration, so that further removal will be necessary before disposing of the stripped waste.

8.1.3.9 Carbon Adsorption—The only available data concerning the removal of nitrosamines from solution have been generated for caustic DMN waste by the Air Force. Isotherms have been obtained for three different activated carbons, with capacities calculated in the range of 1×10^{-3} moles/g to 2.4×10^{-3} moles/g. Column studies using 25 g of carbon have shown that breakthrough at 1 ppm DMN occurs after 0.5 liter of waste containing 218 ppm DMN has been treated. If 1 ppm is an acceptable effluent concentration, carbon requirements would be on the order of 0.5 pound per gallon of this waste. Activated carbon therefore may not be a logistically feasible treatment method for large volumes of DMN wastes. However, even though hard data are lacking, nitrosamines such as nitrosodiphenylamine would be expected to be much more efficiently removed by activated carbon. This method should be studied further for high-molecular-weight nitrosamines.

Disposal of the contaminated activated carbon may present a problem. Incineration of the carbon will result in destruction of the adsorbed nitrosamine but will be extremely expensive because of fuel and carbon costs. Regeneration of the carbon with proper treatment of off-gases may be possible, but no data are available on the efficiency of regeneration or the air pollution hazard associated with this specific technique. Disposal of the spent carbon in a landfill should not be considered because it is very likely that nitrosamines would be leached out of the carbon by rainwater.

8.1.4 Ultimate Disposal Methods for Nitrosamine-Containing Wastes

Even if treatment techniques are successful in destroying or removing nitrosamine in water, the remaining sludges or waste water will not be safe enough for reuse and must be disposed of properly. Several alternative techniques include landfill disposal, deep-well disposal, ocean dumping, and incineration.

8.1.4.1 Landfill Disposal—The nature of landfill disposal is such that it is susceptible to leaching and rainwater runoff. Therefore, before this type of disposal is used for toxic materials, permanent and total detoxification and/or immobilization must be performed. The U.S. Air Force has looked at methods of immobilizing its DMN-contaminated waste water; but, to date, the various gelling and "chem-fixing" techniques have not been successful in totally eliminating leaching of DMN by rainwater. Thus, landfill disposal should not be considered until such time as a satisfactory immobilization technique is developed.

8.1.4.2 Deep-Well Disposal—The technique of disposing of waste into a specially drilled and cased deep well, the location of which has been carefully selected, may be acceptable under special circumstances. In this technique, waste is permanently entombed in porous rock. Deep-well injection has been used to dispose of many toxic wastes with satisfactory results for many years. A few cases, however, have resulted in near disasters when improper drilling techniques were used or when the well was located in a drinking water aquifer or an aquifer that eventually surfaced. Extreme care must be exercised, therefore, before a decision is made to inject nitrosamine waste into a disposal well. This approach is generally not considered acceptable in geologic fault regions.

8.1.4.3 Ocean Dumping—The ocean disposal of nitrosamine wastes, like any other waste, is subject to many restrictions and preconditions. Before disposal, permits must be issued and, prior to their issuance, both public hearings and biological and treatment studies may be required. Ultimately, an environmental impact assessment must be made that compares the impact of ocean dumping

with that of other disposal methods. Since it is unlikely that untreated nitrosamine has no environmental impact, pretreatment will almost certainly be required.

8.1.4.4 Incineration—The incineration of nitrosamine waste is theoretically possible. Studies have shown that nitrosamines, specifically DMN, are decomposed in oxygen at 300°C and above. Although wastes containing these compounds are candidates for incineration, other constituents of the waste may preclude the use of this disposal technique. The high salt content of UDMH rocket fuel waste, for example, may lead to breakout of the refractory lining of an incinerator. Such factors must be considered in selection of materials for incinerator design. It is important, therefore, to ascertain the exact nature of the waste before deciding to incinerate it.

8.1.5 Control of Plant Location

Considering the possibility of nitrosamine formation from the atmospheric reaction of NO_x with secondary and tertiary amines and with compounds containing tertiary amino groups, it might be reasonable to keep separate the effluent streams containing these reactive components.

A control strategy might involve minimizing contact between NO_x -containing plumes and industrial effluent gases containing amines or nitrosamine precursors. This strategy would involve the use of appropriate industrial zoning regulation. While this approach would not completely eliminate the atmospheric formation of nitrosamines, it would reduce it. Since the rate of production of nitrosamines is dependent on the concentration of the reactants, dilution of the reactants before they come in contact will depress the reaction rate.

8.2 TECHNOLOGY FOR CONTROL OF AIR EMISSIONS OF NITROSAMINE PRECURSORS

Secondary amines and nitrous acid or nitric oxides are precursors for the formation of nitrosamines in the vapor phase. Organic decomposition resulting in the amines as a byproduct can yield nitrosamines in the presence of nitrites. Vapor-phase amines can be controlled, but the control of nitric oxides, nitrites, and fine particulates is most difficult. Control methods for these materials are discussed in great detail in the literature, and only a summary is presented here.

8.2.1 Control Technology for Amines

The motivation for control of amine emissions has historically been the control of offensive odors. Because of this objective, the quantification of amine emissions has been in terms of "odor units," and much of the literature defines control efficiency in terms of odor units. Because of the public reaction to offensive odors, high levels of amines have not been emitted into the ambient air.

Wet scrubbing can be used to remove amines from an effluent gas stream. Aqueous solutions of hydrochloric, sulfuric, or sulfamic acids, in concentrations of approximately 5 percent, are used as absorption media. Reductions in the amine concentrations of 90 to 99 percent are reported in a study by Dickerson and Murthy.⁹ Scrubbing is the most economical and efficient method for amine removal from these process streams, which produce large volumes of effluent gas with low concentrations of amines. Amine hydrochloride salts are formed in the case of hydrochloric acid scrubbers and there is the possibility of reaction with nitrite ions to form nitrosamines if the nitrate-nitrite content of the water is high. Extracted amines must be recovered from the water phase. Neutralization and distillation, followed by either recycle or disposal by non-polluting methods (incineration, for example), are required to make this method acceptable.

Incineration or high-temperature oxidation (combustion) destroys amines in the effluent gas phase.¹⁰ The feasibility of this control method depends on the moisture content of the gas phase, the concentration of amines in the effluent gas, and the volume of effluent gas being handled. The cost involved may be prohibitive, given current energy costs. Furthermore, the products of combustion of the effluents should not be overlooked, for in the case of amines they would include oxides of nitrogen that would have to be controlled.

Adsorption on activated charcoal is used effectively to remove organic vapors. The adsorbed organic materials may be eluted with heated air, nitrogen, or an organic solvent; then condensed and recovered. Gas streams must be cooled, since efficient adsorption does not take place at temperatures in excess of 120°F.⁷ The adsorptive capacity of activated charcoals for low-molecular-weight amines is low, so that this method is less effective for those amines that have the capacity of forming the potentially most active carcinogens. Gas streams containing particulate matter must be "cleaned" before passing through the adsorber. This usually means a scrubber-contact condenser. The cost of regeneration of the adsorbent, the relatively low capacity for low-molecular-weight amines, and the necessity for removing particulate matter make this method less interesting than wet scrubbing except in some special circumstances.

Condensation of an organic vapor to its liquid state by cooling is used to purify gas effluents when the effluent has a relatively low moisture content and easily condensable organic vapors. As was mentioned above, this method is usually used in conjunction with other control methods to recover adsorbed organic materials and to reduce the moisture content of the effluent gases to be incinerated. For low-molecular-weight organics, this method will only be of limited value since 100 percent removal cannot be attained.

8.2.2 Control Technology for Nitrogen Oxides

Nitrogen oxides control is difficult. Summarized, the simplest modification is control of the type of fuel burned; but, unfortunately, the most desirable fuel for the control of NO_x emissions is the least available and the most expensive. Process modifications resulting in lower flame temperatures and a minimum of excess air for combustion give reduced NO_x emissions. These modifications may eventually be adopted even though the use of minimum excess air may result in the increased emission of carbon monoxide and particulate matter.

Catalytic reduction of NO_x on noble metal catalysts is feasible technically but is limited by the presence of other more easily reduced species in effluent gases, making the economics of the process unfavorable for most stationary combustion sources. The development of non-noble metal catalysts is being pursued and could make this a practical method of control at some future date.

Aqueous scrubbers are not effective for NO_x control because nitric oxide has very low water solubility. Oxidative solutions have been used but have not resulted in an economically acceptable reduction of NO_x to the desired levels. The presence of other more easily oxidized species in the effluent gas requires excessive concentrations of oxidants and makes the economics of this control method impractical.

Exhaust recycle can provide some control for NO_x emissions from the internal combustion engine,⁸ but power is reduced and operating costs are increased. Therefore, this method has not been widely adopted.

The absorption of nitric oxide emissions in organic solvents or on polymeric adsorbents is currently under investigation. There are no short-range solutions expected and the potential of a long-range solution is uncertain.

8.2.3 Control Technology for Particulates

Many nitrosamines and their precursors have relatively high melting and boiling points and do not exist in the gas phase. They may be omitted in some processes as dust or as aerosols. Dusts, whether consisting of finely divided particles of nitrosamine precursors or of inert materials upon which precursors are adsorbed, have the potential for reacting with nitrogen oxides in the atmosphere. The particulates, therefore, must be removed from the effluent gas and disposed of safely. The usual particulate removal technologies are applicable and are summarized here.

Bag filters are the most efficient method for the removal of particulates from effluent gas. The nature and size of the particle will determine the filter material, and the efficiency of the process will depend on the velocity, temperature, pH, and moisture content of the stream, and the chemical nature of the gas and particulates.¹¹

Wet scrubbing is a reasonably economical and efficient method of removing relatively large particulates from effluent gas streams. As the particle size decreases, energy requirements and, therefore, cost increase until the cost exceeds that of fabric filtration or of electrostatic separation. Disposal of the contaminated water presents a problem and militates against the use of wet scrubbing.

Electrostatic precipitation of dust particles is an efficient method of control. The initial cost is the highest of any method of control, however, and for this reason electrostatic precipitation would not be the control method of choice except where other methods are inapplicable for some reason.

Inertial separators are often used to collect particulate matter. The efficiency of collection is not high for small particles, and the method is not recommended for toxic particulates because total removal cannot be attained.

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